

BIOLOGICAL
REVIEWS
AND
BIOLOGICAL PROCEEDINGS
OF THE
Cambridge Philosophical Society

Edited
by
H. MUNRO FOX
and
PUBLISHED QUARTERLY

VOLUME V
1930

CAMBRIDGE
AT THE UNIVERSITY PRESS
1930

PRINTED IN GREAT BRITAIN

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THE FORMATION OF GERM LAYERS IN INSECTS

By L. E. S. EASTHAM (CAMBRIDGE).

(Received July 24, 1929.)

(With Two Text-figures and One Table.)

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I. INTRODUCTION.

No matter to what zoologist must be given the credit for formulating the germ-layer theory, it is really to him that we must turn for the origin of the mass of controversy which for the last sixty years has focussed on the process of gastrulation in insects. Prior to the time of Wolff, 1759, the "emboîtement" theory of development held the field which maintained that either the sperm was the miniature individual which found favourable soil for growth in the parent or egg, or the egg was the individual stimulated to growth by the sperm. In 1759 Wolff countered this theory with that of Epigenesis, but not till 1828 did Wolff's findings gain any acceptance. The well-known embryological work of Von Baer (1828) confirmed Wolff's doctrine of the appearance of layer-like anlagen in a developing embryo from which layer the organs arose. Von Baer laid down the general principle that for the correct basis of

classification comparative embryology is indispensable. On this account it is he rather than Wolff who is now generally regarded as the founder of the germ layer theory. It must however be pointed out that Wolff himself had no true conception of the process of layer formation as the first necessary step in all differentiation, and Von Baer himself assigns the credit for the discovery of germ layers to Pander, who contributed one of the earliest accounts on the embryology of the chick in 1818. Von Baer states that Pander through his clear recognition of the splitting of the germ—a process which remained obscure to Wolff—shed a light on all forms of development (see Russell, 1916). It is not intended to trace the development of embryological study from these early times to the time when insect development was first seriously taken up. It is sufficient to point out that the authors belonged to the pre-evolutionary period and that it was not till after Darwin had presented his theory of the origin of species by natural selection, 1859, that embryology was to be taken as one of the most important criteria for establishing relationships and for proving evolution. Haeckel's biogenetic law, 1877, which states that Ontogeny is immediately conditioned by Phylogeny, that it is a short rapid recapitulation of phylogeny conditioned by the physiological functions of heredity and adaptation, following Darwin's theory of evolution, led embryologists to search in the development of individuals for stages which could be regarded as ancestral. So we find in standard text-books of embryology statements to the effect that "the blastula and gastrula stages at the end of cleavage represent forms which are ancestral to all metazoa and that one is justified in the assumption that in these two stages there exists a repetition of ancestral forms which are common to all metazoa" (Korschelt and Heider, 1895). MacBride, 1915, in stating the biogenetic law as follows, "The individual in its development recapitulates the development of the race," remarks that if this law can be substantiated the interest in embryology becomes immense, it binds all the innumerable phenomena of development into one coherent scheme and opens the door to the hope that we may yet be able to sketch the history of life on the earth.... It is therefore the essence of comparative embryology to separate the fundamental ancestral traits of development from the superficial and secondary. Both Huxley (1877) and Lankester (1900) with Haeckel, saw in the gastrula a form common to all metazoan embryos. Brachet (1921) criticises this common attitude and points out that much labour has been wasted and much time lost in the search for a state similar to the gastrula. Though there may be no gastrula in the Haeckelian sense in the development of certain forms, Brachet maintains that all developmental conditions are bound together by a thread "which is easy to find when one looks for it." The importance of the gastrula as a solid foundation for the study and interpretations of complicated developments is recognised, but its importance from the point of view of embryology is independent of its phylogenetic significance. Whether we adopt the older attitude and follow Haeckel, or the newer attitude and follow Brachet, the interest in the process of gastrulation in insects will remain the same.

It is generally accepted that arthropods represent an evolution of the annelidan type of structure. Since the early stages of development of all arthropods are very

different from those in the development of annelids, it is natural to look to the constitution of the eggs in these two phyla for the cause of this difference. The prime difference lies in relative amounts of contained yolk—the annelidan egg being comparatively non-yolky or alecithal, the arthropod egg being centrolecithal, loaded with yolk which is surrounded by a coating of comparatively yolk-free protoplasm. The difference in amount and distribution in yolk is paralleled in the case of *Amphioxus* and the chick, and yolk plays no less a part in modifying the course of development in the arthropod than it does in modifying the course of development in the Amphibia, Reptilia and Aves. But whereas in vertebrate embryology there appears to have been established a general uniformity of opinion concerning the developmental process (having regard to the distorting influences), in the case of arthropods and particularly insects no such commonly accepted opinion seems to exist. No matter what the reason may be, the early organisation of an insectan embryo and the path by which the differentiation into layers is attained present considerable difficulties to the embryologist who tries to establish uniformity of development between insects and other coelomate invertebrates. Further, such difference of opinion exists as to the course of events proceeding in the eggs of insects of the several orders that one can heartily agree with Weismann (1863) when he says that nowhere in the animal kingdom is the anatomy so distorted as in the insects, and scarcely anywhere else are the germ layers so difficult to recognise as here.

II. OUTLINE OF EARLY INSECT DEVELOPMENT.

The typical insect egg is yolky, and, though it need not be elongated, the embryonic rudiment is generally long at its first appearance and these two factors, yolk and elongation (the latter possibly resulting from the former), would appear to be important in causing modification of the normal process of gastrulation. With the exception, perhaps, of the Collembola and the eggs of parasitic Hymenoptera where yolk is present to a less degree than elsewhere, cleavage of the insect egg is incomplete. Cleavage nuclei, forming with their containing cytoplasm a syncytium ramifying through the yolk, wander to the peripheral cytoplasm to form an outer layer of cells or blastoderm. A ventral thickening of this blastoderm segregates embryonic from extra-embryonic blastoderm. The ventral embryonic plate gives rise to the primary layers and growth is effected at the expense of the yolk. In its relation to the yolk it resembles an inverted chick embryo, but, as might of course be expected, its method of differentiation is highly different. In the insect egg, e.g. typical Diptera, Coleoptera, Orthoptera, Hymenoptera, and Lepidoptera, certain cells along the middle line of this embryonic rudiment pass in towards the yolk either singly or *en masse* and become enclosed against the yolk band (Fig. 1 C'). By common consent the ectoderm now left outside, the enclosed mesoderm and the elongated pore formed during the passing inwards of the latter are homologous with the ectoderm, mesoderm and blastopore of other animals. The main difficulty—about which controversy has raged for the last half century—concerns the endoderm. The alimentary canal of insects as of all arthropods is well defined into three regions—an anterior

ectodermal part or stomodaeum, a corresponding posterior proctodaeum, and a median portion which is not lined with chitin and most conveniently (if not

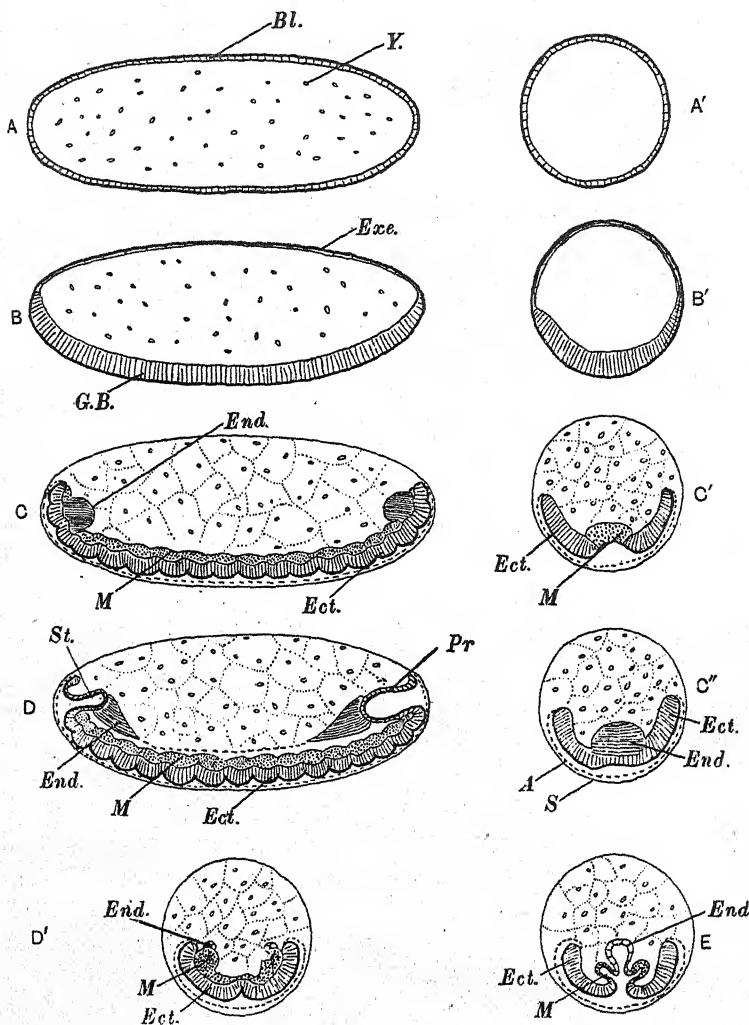


Fig. 1. To illustrate the main features of insect embryology. A, B, C, sagittal sections. A', B', C', transverse sections through corresponding stages. D, sagittal section of embryo with germ layers present. C'', transverse section through C stage embryo in region of future mouth. D', transverse section through D stage embryo. E, transverse section through posterior endodermal groove of muscid embryo (after Nusbaum and Fulinski). A, amnion; Bl., blastoderm; Ect., ectoderm; End., endoderm; Exe., extra-embryonic blastoderm; GB, germ band; M, mesoderm; Y, yolk nuclei; S, serosa; St, stomodaeum; Pr, proctodaeum.

generally) regarded as endodermal. But since difference of opinion still exists as to whether the mid-gut arises from the endodermal germ layer or not, I shall speak of the tissue from which the mid-gut arises not as endoderm but as mesenteron rudiment.

By nearly common consent (certainly common consent of modern investigators) the mesenteron rudiments first appear at both ends of the embryo in the regions of the stomodaeum and proctodaeum. These two rudiments grow towards each other (backwards and forwards respectively) between the enclosed mesoderm and the yolk, as paired longitudinal strands which by growth on their median or inner sides, unite to form a sheet of cells on which rests the yolk. Whether such tissue is endoderm or no, we now have in the embryo as shown in the diagram, the primordia from which the main organs of the body are to develop. From the ectoderm arises the body wall, nerves, tracheæ, stomodaeum and proctodaeum. From the mesoderm arise muscles, germ cells, fat body, cardioblasts, and from the mesenteron rudiments we get the lining of the larval mid-gut.

III. THEORIES ON INSECT GERM LAYER FORMATION.

Before reviewing the gastrulation process in the various orders of insects it will be profitable to tabulate the main views which exist concerning its interpretation.

(1) No true endoderm exists in the post embryonic stages of insects.

It is represented in the embryo only by the yolk nuclei. The mesenteron rudiments are inward proliferations from the ends of the stomodaeum and proctodaeum and therefore deemed ectodermal.

(2) No true endoderm exists in insects (as above). It is represented in the embryo by certain cells which migrate from the rudiment to the yolk.

(3) The whole mass of cells enclosed at gastrulation (see p. 3) is partly mesodermal and partly endodermal—endodermal at the two ends where it forms the mesenteron rudiments and mesodermal in the other parts.

(4) A true endodermal mid-gut exists and is derived from yolk cells; the yolk nuclei and surrounding yolk on this theory representing the third germ layer differentiated at gastrulation or mesoderm formation.

(5) A true endodermal mid-gut exists and is derived from anterior and posterior rudiments which arise by ingrowth of cells from the germ band independent of the mesoderm. The endoderm has therefore a bipolar origin.

(6) As view No. (5) with the addition that the link between the two rudiments is embryonic and transient.

A consideration of the above views will at once indicate how closely the problem is wrapped up with the germ-layer theory. Each investigator in turn seems to have been largely influenced by the desire to refer one stage of insectan development to a normal blastula and a later one to a normal gastrula. In many cases it seems clear that want of agreement rests on differences of interpretation rather than differences of concrete results obtained, which is much to be deplored. As an example we may quote the case of the development of *Apis*. Bütschli (1870) derived the mesenteron from yolk cells. Grassi (1884) derived it from anterior and posterior sections of the mes-entoderm, as did also Pentrunkewitsch (1902). In 1871 Kowalevski stated that the mesenteron arose from cells which were delaminated from the splanchnic layer of the mesenteron, while Nelson (1915) shows that it

arises from two cell masses, anterior and posterior—which are quite distinct from the mesoderm. Yet, with the exception of Bütschli (1870), there seems to be little difference in the actual findings of these several investigators. It must be admitted, however, that many views are based on very superficial study, or study by imperfect methods of technique, so that, before any sound conclusions can be arrived at, the highest kind of research is required, with the employment of the most precise cytological methods and, to quote Nelson (1915), "an eye single to the facts and regardless of preconceived theoretical considerations."

IV. INSECT ORDERS.

APTERYGOTA.

Of the orders of apterygote insects there is no account of the embryology of the *Protura*.

(a) *Collembola*.

Uzel (1898), Claypole (1898), Uljanin (1874), Provazek (1900), and Philiptschenko (1912), have contributed to our knowledge on the embryology of the *Collembola*. The eggs of these animals are peculiar among insects in that they are holoblastic. *Anurida maritima*, the subject of study by Claypole (1898), may be taken as an example. Holoblastic cleavage leads to the formation of a morula. Then follows a migration of nuclei and cytoplasm to the surface, leaving a middle mass of yolk. The blastoderm is thus formed by outward migration to the egg periphery. Oblique mitosis now occurs in the cells forming this peripheral layer with the result that an inner layer of mesoderm is formed by a process of multipolar immigration. A central mass of cells distinct from ectoderm, mesoderm, or yolk nuclei was described by her as endoderm. This group of cells divides into two and the two parts come to lie in the region of the stomodaeum and proctodaeum and subsequently form longitudinal strands, the anlagen of the mid-gut. Uljanin and Uzel agree with Claypole, but Provazek, differing rather in interpretation than in actual results, calls the central cell mass yolk cells.

His conception therefore of the layers formed is that the blastoderm at its first formation is epiblast, the contained yolk with its nuclei, the hypoblast (endoderm) and the mesoblast occurs later in the manner described, from the epiblast. Uljanin, Uzel and Claypole distinguish between endoderm cells and yolk nuclei in the yolk, which they maintain can be distinguished from each other cytologically. This point is of importance, since Heymons (1895), the champion of the view that no true endoderm exists in larval or adult Pterygota, gains much support by declaring that apterygote insects develop the mid-gut from yolk nuclei.

In 1912 Philiptschenko described the embryonic developments of *Isotoma cinerea*. His is the only modern investigation on any member of the *Collembola*, and, while agreeing with the above authors on the origin of the mesoderm by multipolar immigration from the blastoderm, he clearly removes one of the mainstays of the Heymons theory by his clear and convincing description of the development of the endoderm. He maintains that previous workers have mistaken the germ cells

for endoderm and that the cell groupings near the stomodaeum and proctodaeum are not derived from an original mass of cells in the yolk but from cells which pass inwards from the blastoderm at the time that the mesoderm is similarly being formed. He maintains that the mid-gut in the Collembola and probably in all Apterygota is differentiated from the lower layer or mesoderm in three parts, an anterior and posterior, and a median diffuse. Further reference will be made to this.

(b) *Thysanura.*

Heymons (1896*b*, 1897*a*, 1897*b*), and Uzel (1897*a*, 1897*b*), have investigated the Thysanura. Both in *Campodea staphylinis* and *Lepisma saccharina*, the subjects for study, there is a close agreement with the development of pterygote insects. In the first place cleavage is not total, and blastoderm formation and that of a ventral embryonic rudiment are as in the Pterygota. Mesoderm is formed by immigration from the rudiment and, according to Heymons, it is the yolk nuclei which forms the lining of the mid-gut, the contained yolk gradually being absorbed as differentiation proceeds. Uzel, on the other hand, maintains in *Campodea* that endoderm cells originate in the germ band and not in the yolk, and believes the same to be the case in *Lepisma*. In both Uzel's and Heymons' accounts there is lack of continuity, which, in view of Philiptschenco's findings in the Collembola, renders further careful investigations on these forms highly desirable. It ought to be pointed out that the findings of Heymons in *Lepisma* with regard to the origin of the mid-gut from yolk nuclei undoubtedly influenced him in his interpretation of the germ layers of the Pterygota.

PTERYGOTA.

(c) *Orthoptera and Dermaptera.*

Rathke, H. (1829), Ayers (1884), Patten (1884), Korotneff (1885), Nusbaum (1886), Cholodkovsky (1888, 1890 and 1891), Wheeler (1889 and 1893), Gruber (1890, 1891*a* and 1891*b*), Heymons (1895 and 1897*a*), Viallanes (1891), Nusbaum and Fulinski (1906 and 1909), Rabito (1898), Hammerschmidt (1910), Strindberg (1914), Leuzinger, Wiesmann and Lehmann (1926). Perhaps the number of investigators into the development of the orthopteran egg may be taken as an indication of the degree of difference in germ-layer formation. Cleavage in all cases is incomplete and the blastoderm is formed in the usual way from the products of cleavage. From the embryonic rudiment which develops on the ventral side of the blastoderm, mesoderm is formed from the cells which are budded inwards towards the yolk. In this the Orthoptera resemble the Apterygota. There is however a big difference in the area from which such cell migration takes place. In *Blatta*, Wheeler (1889), there is a small blastoporal area restricted to the future posterior end of the embryo from which cells are budded inwards and passed forwards between the germ band and the yolk. Bruce (1887) finds a gastrular groove in Mantidae, as also does Gruber (1891*a* and *b*), in the Acrididae, and cells are budded inwards from the whole length of this. In the Phasmidae, Tettigonidae and Gryllidae (see Ayers, 1884 and Korotneff, 1885) mesoderm is formed by a process of multipolar immigration from any part of the inner surface of the germ band. It is difficult to

believe that the nearly related families of the well-defined order Orthoptera did not all at one time possess the same method of lower layer formation. There is even now a strong resemblance in the processes, namely that in all forms mesoderm is formed by proliferation. Does the distributed or restricted area of proliferation represent the primitive condition? In more specialised insects mesoderm formation is restricted to the middle line and one is tempted to the conclusion that such a restricted area of mesoderm formation is a recent acquirement. It is worthy of note that in the endopterygote insects, though mesoderm formation is restricted to the middle line of the rudiment, cells are commonly budded into the yolk from other parts of the germ band, a process which is perhaps a vestige of the original multipolar immigration origin of mesoderm in Apterygota. If this is so, how does *Blatta* with its restricted blastoporal pit fit in the scheme? Wheeler (1889) suggests two alternatives: (1) that it is a degenerate process derived from that of the higher insects with an elongated blastopore as in the Coleoptera (see later), or (2) that the blastopore as in *Peripatus*, Sedgwick (1885-1888), dividing to form mouth and anus, becomes greatly stretched out in higher insects, leaving mesoderm formation along the whole length of the embryo. *Blatta* on this view is regarded as representing an intermediate condition before the great separation of anterior and posterior ends occurred. This may be better understood when it is pointed out that gastrulation in *Blatta* begins when the embryonic rudiment is very small. Mesoderm formation can therefore take place from a small restricted area in this insect, while in higher forms it is retarded till the germ band is formed along the whole length of the egg. The first view of Wheeler is difficult to accept, since it infers that *Blatta* does not occupy the generalised position which its adult anatomy clearly indicates. The second view is the more sound (and it is interesting to note that the factor of retardation enters into the problem, a factor which, as will be seen later, has an important bearing on the removal of many obstacles to the establishment of uniformity of opinion and interpretation of the general process of gastrulation in insects) and it is interesting to note that Nusbaum and Fulinski (1909) in *Gryllotapa vulgaris* find a long germ band and, from the whole middle line of this, cells pass inwards, the majority of which form the mesoderm. As the embryo increases in length the median position is faintly depressed inwards towards the yolk—a condition suggestive of gastrulation as seen in endopterygote insects. The next important feature in gastrulation of the Orthoptera is the formation of endoderm. In this order the most work has been done by Heymons (1894, 1895 and 1897). His very definite conclusions on the origin of definitive mesenteron from cells proliferated from the blind ends of the stomodaeum and proctodaeum are quoted by every investigator into these questions and the idea that the insectan mid-gut is ectodermal is inseparably associated with his name. He has many supporters, e.g. Rabito (1898), Leuzinger, Wiesmann and Lehmann (1926). Among earlier workers who arrived at the same conclusions may be mentioned Graber (1891 *a* and 1891 *b*), Korotneff (1894). It is here, however, that we meet for the first time the main differences of opinion as to the significance of the cells from which originates the mid-gut. Thus Ayers (1884) found that the mid-gut arose from yolk cells. Korotneff (1885) describes endoderm as originating

in yolk cells. These however did not form the mid-gut, the latter being differentiated from blood cells. The early work of Graber (1888) on *Stenobothrus* and that of Cholodkowsky (1888 and 1889), and the more recent works of Hammerschmidt (1910) and Strindberg (1914) give a splanchnic mesoderm origin for the definite endoderm. In view of the many times that yolk cell and splanchnic mesodermal origin have been refuted, these may be left out of consideration. The only view of real merit, counter to that of Heymons, is that of bipolar origin of endoderm from rudiments proliferated inwards from the germ band in the anterior and posterior regions. In this connection must be mentioned the convincing work of Wheeler (1889 and 1893) and Nusbaum and Fulinski (1906 and 1909). The latter workers give very convincing figures showing the formation of endodermal rudiments before the invaginations of the stomodaeum and proctodaeum develop. Leuzinger, Wiesmann and Lehmann (1926), on the other hand, show in *Carausias* stomodaeal and proctodaeal invaginations from the blind ends of which the mesenteron rudiments pass inwards, i.e. the mid-gut is entodermal in origin.

Strindberg (1914) deserves mention here in that he finds that the tissue which passes inwards from the germ band to lie against the yolk has a dual constitution, i.e. it is ento-mesoderm. From the whole length of the embryo endoderm is formed by delamination from the ento-mesoderm. His work does not carry the mark of completeness which characterises that of Leuzinger, Wiesmann and Lehmann (1926).

(d) *Isoptera*.

Knower (1900), Strindberg (1913).

From the small amount of work which has been done on the embryology of termites it appears that there is little difference between the early stages of egg development here and those of the Orthoptera. Knower was unable to find any gastrular invagination, and mesoderm was formed in *Eutermes (rippertii?)* by multipolar immigration. Strindberg is able to confirm this and adds that the endoderm is formed by delamination from the mesoderm.

(e) *Odonata*.

Brandt (1869), Tschuproff (1903), Heymons (1896 a). The most recent worker on Libellulid development, Tschuproff, finds that mesoderm arises from a longitudinal gastrular groove. The important feature in her work lies in the statement that the mid-gut of the nymph arises in three sections, a median section arising from the yolk cells which take on the form of an epithelium, an anterior portion derived from the stomodaeum and the posterior from the proctodaeum. This is regarded by this author as forming an important intermediate condition between the Apterigota and Pterygota in that, at the time of writing, Heymons' theory on the ectodermal origin of the mid-gut in pterygotes held the field and that the mid-gut of apterygotes was thought to arise from yolk cells. The later work of Philitschenko (1912) already referred to is important to bear in mind here, since he showed that, in the Collembola, yolk cells took no part in endoderm formation. Tschuproff's findings require confirmation before further serious comment can be made on them.

(f) *Hemiptera.*

Zacharias (1884), Tannreuther (1907), Karawaiew (1893), Will (1888 *a*, 1888 *b*), Witlaczil (1884), Brandt (1869), Gruber (1878, 1889), Heymons (1899), Seidel (1924). Witlaczil made only a superficial examination of aphid development and found that the mesoderm arose by multipolar immigration. No gastrular furrow was observed. Will, on the other hand, finds a distinct longitudinal gastrular furrow along which mesoderm arises. Endoderm cells are first passed inwards from this region into the yolk and later collect in two regions anteriorly and posteriorly in the regions of the stomodaeum and proctodaeum. Gruber (1878 and 1889) also finds a distinct elongate groove or blastopore. The most important work is that of Seidel (1924) on *Pyrhocoris apterus* L. Here it is found that ento-mesoderm is formed along a longitudinal blastopore and that anterior and posterior ends of this give rise to the elements from which the mid-gut is formed. This confirms Karawaiew (1893).

(g) *Neuroptera.*

Tichomirowa, O. (1892 and 1890). We have the most meagre account of this phase of insect development in the Neuroptera and the observations are so peculiar as to warrant further work being done on this interesting order. Tichomirowa finds that the blastoderm arises from the cleavage cells. Some of these remain behind in the yolk, later to apply themselves against the inner surface of the blastoderm so forming mesoderm. The cells first passed into the yolk are called primary endoderm.

(h) *Trichoptera.*

Patten (1884), Brandt (1880), Kowalevski (1871), Zaddach (1854). In his work on the embryology of *Phryganids*, Patten finds an undoubted gastrular furrow—an investigation of the germ band starting posteriorly and ending in the region where the mouth will subsequently develop. The groove so formed closes from behind forwards and in this way a longitudinal mass of cells is enclosed between the germ band and the yolk. This is the mesoderm. Endoderm is described as arising by delamination from any point in the blastoderm. From such "yolk cells" Patten maintains that the mid-gut is formed. The very early work of Zaddach (1854) on Phryganid development ought to be mentioned here. He appears to have seen the formation of the longitudinal blastopore with its consequent differentiation of mesoderm. But since he confused the gastrular invagination with the neural furrow he made the surprising statement that from the inner layer (mesoderm) both nerves and muscles were differentiated. In view of the many times that origin of the mid-gut from yolk cells has been proved wrong, it is almost certain that Patten's conclusions on this point are incorrect.

(i) *Lepidoptera.*

Bobretzky (1878), Ganin (1869), Gruber (1890), Hatschek (1877), Hirschler (1905), Schwangart (1904), Schwartz (1899), Tichomiroff (1879 and 1882), Toyama (1902), Woodworth (1889), Kowalevski (1871), Tichomiroff (1892), Strindberg (1915), Eastham (1927). The germ band of the Lepidoptera is comparatively long

when gastrulation begins. In the production of mesoderm from the germ band the process is admittedly similar to that found by Patten in the Phryganid *Neophylax* (1884). A middle portion of the germ band is first differentiated from two lateral plates and these later enclose the middle plates by a process of overgrowth with the resultant formation of ectoderm and mesoderm. Kowalevski (1871) described the overgrowth of the middle by the later plates. Bobretzky (1878) noticed in addition a proliferation of individual cells. In 1890 Gruber was the first to notice that as well as the overgrowth process the middle plate became invaginated toward the yolk in a manner similar to that found in Trichoptera by Patten (1884). Subsequent workers, Schwartze (1899), Toyama (1902) and Schwangart (1904) conclude that the process occurs differently in different regions of the embryo, cell proliferation in one part, invagination in typical gastrular fashion in another and overgrowth without invagination in a third region. Gastrulation in Lepidoptera for these authors is therefore bound to no fixed scheme.

In 1927 Eastham shows in *Pieris* that what the above authors saw in separate regions are in actual fact separate processes which the germ band undergoes in sequence. Thus the germ band of uniform thickness proliferates loose cells from its median portion and the thin middle plate then becomes slightly invaginated. Finally the lateral plates grow inwards to enclose the slightly invaginated middle plate. The interest in this lies in the fact that each of these processes has some resemblance to the process in certain other insects. Thus the first proliferation of cells resembles that found in Orthoptera and Isoptera. The invagination is similar to that found in Trichoptera and Coleoptera and the final process of overgrowth is the same as that described by most authors in the Hymenoptera. In the matter of endoderm formation much difference of opinion exists. Tichomiroff (1882 and 1892) maintained that this originated from yolk cells, while in 1879 he stated that it arose by delamination from splanchnic mesoderm. In 1878 Bobretzky assumed that endoderm had its origin in yolk cells. Later Schwartze (1899) and Toyama (1902) find that the mid-gut arises from stomodaeal and proctodaeal invaginations and with Heymons (1895) (Orthoptera) maintain that larval and adult insects possess no organ which has its origin in true endoderm. Hirschler (1905) agrees with them in that the mid-gut arises as cell proliferations from the blind ends of the stomodaeum and proctodaeum respectively. He also adds that the mid-gut partly develops from the median section of the lower layer (mesoderm). This latter statement is most probably incorrect since the median portion of the mesoderm almost universally gives rise to blood cells and the proliferation of cells for this purpose may easily have been wrongly interpreted.

Strindberg (1915) in *Bombyx* found distinct anterior and posterior endodermal rudiments in front of and behind the stomodaeum and proctodaeum respectively and give rise to the mid-gut. Eastham (1927) confirms this.

(j) *Coleoptera.*

Lecaillon (1897a, 1897b, 1898), Deegener (1900), Czerski (1910), Friederichs (1906), Hirschler (1909a, 1909b), Saling (1907), Heider (1885 and 1889), Nusbaum

(1888), Tichomiroff (1890), Voeltzkow (1889*b*), Wheeler (1889), Mansour (1927), Graber (1891 *a* and *b*), Strindberg (1913). There seems to be little difference of opinion among the above mentioned workers concerning the origin of the mesoderm. In Coleopterous embryos there commonly occurs a longitudinal invagination of the middle part of the germ band to form a tube. This breaks down into an irregular layer of cells between the ectoderm and the yolk to form the mesoderm in which mesoblastic somites ultimately form. Concerning the origin of the mid-gut however there is much controversy. Thus Tichomiroff (1890) maintains that it develops from yolk cells. This, as in most other such cases subsequently investigated, may almost certainly be taken as an error of interpretation due to an imperfect technique. The two main views sharply opposed to each other are: (*a*) there is no true endoderm in the fully formed embryo mid-gut arising from cell proliferations at the blind ends of the stomodaeum and proctodaeum respectively; (*b*) true endoderm arises at anterior and posterior ends of the embryo irrespective of the stomodaeum and proctodaeum and from this the definite mid-gut develops. The two schools are sharply defined. To the first belong Lecaillon, Deegener, Czerski, Friederichs, Saling, Voeltzkow, Graber and Mansour, while to the second we find contributions from Wheeler, Hirschler and Heider.

A minor modification of the latter view is found in the works of Hirschler (1909), Heider (1885), Kowalevski (1871) and Nusbaum (1888), these authors finding that, in addition, the splanchnic layer of the mesoderm contributes to the formation of the mid-gut.

That some of the differences of opinion rest on interpretation rather than actual results is certain. As an example may be quoted the works of Hirschler (1909) and Friederichs (1906). Hirschler describes development of *endoderm* cells which eventually lie between stomodaeum and proctodaeum respectively and the yolk. Friederichs' description is almost identical with this, but he prefers to regard such cells not as endoderm but as ectoderm.

Undoubtedly the most important embryological work dealing with this phase of development is that of Mansour, on the development of the larval and adult mid-gut of *Calandra oryzae*. Mansour regards the blastoderm stage as the blastula, and yolk cells form a secondary feature of no germ layer significance. The ventral groove which develops at mesoderm formation is a gastrular furrow. The walls of this groove consist of mesodermal and endodermal elements but this loses its endodermal part by proliferating cells into the yolk which form no definite larval tissue, and the remaining cells resulting from the invagination are mesoderm only. The mesenteron is entirely ectodermal arising from stomodaeum and proctodaeum. Mansour is dissatisfied with the reality of the anterior endoderm rudiment described by Wheeler in *Doryphora* (1889) and maintains that Wheeler's posterior endoderm rudiment is nothing more than the genital rudiment. He follows his point to the end by proving that the adult mid-gut at metamorphosis is not formed from replacement cells in the wall of the mid-gut but from elements arising in the posterior end of the metamorphosing fore-gut and therefore is ectodermal. But since the larval mid-gut is, according to him, ectodermal, the fact that replacement cells in

the larval mid-gut do not contribute to the formation of the adult mesenteron loses its significance.

(k) *Diptera*.

Bütschli, O. (1888), Escherich (1900 *a*, 1900 *b*, 1901), Gruber (1889), Kowalevski (1886), Ritter (1890), Voeltzkow (1888), Weismann (1863), Pratt (1900), Noack (1901), Schmidt (1889).

Kowalevski (1886), describing the embryonic development of muscids, found a very elongated germ band which extends from the ventral to the dorsal side at the two poles. The middle portion of this germ band becomes invaginated to form a tube from the walls of which mesoderm is formed. Behind and in front, the middle portion of this invaginated tissue becomes distinct from the remainder and forms endoderm from which develops the mid-gut. Bütschli (1888) confirms this, as also do Schmidt (1889), Voeltzkow (1888), Ritter (1890) and Escherich (1900 *a*, 1900 *b*, 1901). The invaginated tissue is therefore called mes-entoderm from which endoderm is only formed at the two ends. Noack (1901) is the most recent writer on muscid embryology and he gives a clear account of germ layer differentiation which is confirmatory of the above views. His account may be summarised as follows. The gastrulation furrow remains widely open in front for a time and the anterior border to the furrow is formed by a layer from which proliferate cells which constitute an anterior endoderm rudiment. As differentiation and closure of the gastrular groove proceed the endoderm anlage begins to grow into a well defined mass of cells projecting into the front end of the gastrular furrow. The mesoderm thereby comes to lie above the endoderm which has meanwhile become enclosed by the gastrular tube. The close contact between mesoderm and endoderm at this point undoubtedly obscured the process of differentiation to the early workers and led them to believe that endoderm was merely an anterior part of the mesoderm. Posteriorly a similar process occurs. It is important to notice that in both cases, endoderm destined to form mid-gut is differentiated *before* the ectodermal invaginations of fore- and hind-gut have begun to develop. Both anteriorly and posteriorly (particularly posteriorly) a condition obtains which strongly suggests annelidan gastrulation. A deep invagination with a definite lumen is found projecting into the yolk. This invagination is bounded by endoderm at its deepest point, and by mesoderm at this junction of the invagination with ectoderm. A separation of these layers is later effected. The mesoderm separates to each side to form somites, the endoderm becomes diffused and by further growth gives rise to two strings of cells which, e.g. in the case of the posterior ends, wander forwards beneath the yolk and above the mesoderm to form the mid-gut rudiments. It is this condition in muscid embryos which has led a number of investigators, Bütschli, Kowalevski, Heider (1885) to the view that insect gastrulation is not so different from the corresponding process in non-yolky eggs of invertebrates and only differs in that the blastula is lengthy, with the result that endoderm has ceased to be formed except at the two ends.

Voeltzkow (1889 *a*) differs from the above authors in that he maintains that the two mid-gut rudiments originate in the ectoderm of the stomodaeum and procto-

daeum. Pratt (1900) also in her account of the embryology of *Melophagus ovinus* finds an ectodermal origin for the mid-gut rudiments. Graber (1889) compromises and finds an anterior endodermal rudiment, but posteriorly cells grow from the proctodaeal wall to form the posterior part of the mid-gut.

(l) *Hymenoptera.*

Bütschli (1870), Carrière (1890), Carrière and Bürger (1897), Dickel (1904), Ganin (1869), Grassi (1884), Kulagin (1892 and 1897), Tanquary (1913), Nelson (1911 and 1915), Kowalevski (1871), Strindberg (1913), Petrunkevitsch (1902). Bütschli thought that in *Apis* the mesenteron originated by "free cell formation," (of nuclei from a protoplasmic matrix). This is obviously impossible. Kowalevski (1871) derived the mesenteron from the splanchnic layer of the mesoderm. Grassi (1884), Petrunkevitsch (1902), and Kulagin (1892 and 1897), regarded the whole mass of cells enclosed against the yolk as mes-endoderm and did not clearly differentiate between mesoderm and entoderm at the two poles. Carrière and Bürger (1897) described the formation of two rudiments by proliferation of cells from each end of the germ band in *Chalicodoma*. Dickel (1904), working at *Apis*, says that endoderm from which the mid-gut develops is present in the form of a discoid mass at the anterior end of the egg in early stages of development. This mass has its origin in yolk cells. Strindberg (1913) examined a number of forms among which were the two ants *Camponotus* and *Formica*. In the latter two forms cells are seen to wander inwards from any part of the germ band to form a layer between the latter and the yolk. This constitutes the endoderm. The middle region of the germ band now suffers invagination to form mesoderm. It is to Nelson (1911 and 1915) that we must turn for the clearest account of Hymenopteron development (*Apis*). When the ventral plate is defined two nearly parallel furrows develop, one at each side of the middle line. These mark the boundaries between a median and two lateral plates. The two laterals destined to form ectoderm gradually grow inwards over the edges of the middle plate. This is thereby enclosed against the yolk as mesoderm. In the anterior parts of the embryo an area of cell proliferation develops with the result that a roundish mass of cells confluent with the mesoderm comes to lie in the region of the future mouth. This is the anterior endodermal rudiment from which the anterior part of the mid-gut develops. At the posterior end of the germ band a fundamentally similar process results in the formation of a posterior endoderm rudiment which contributes to the formation of the mid-gut posteriorly. Nelson shows that the discoid mass of cells described by Dickel is a structure (yolk-plug) which has no connection whatever with the endoderm or mid-gut. He briefly discusses the merits and demerits of the various views with special reference to *Apis*. While discounting the view that the mid-gut is ectodermal, he regards *Apis* as too specialised a form on which to come to any decision as to the significance of this type of development in connection with gastrulation. The mesenteron rudiments may equally be regarded as mesodermal (since both they and the mesoderm arise by the same general process of inward immigration), or as blastodermal since their manner of formation differs in detail from that of the mesoderm. Much de-

pends on the theoretical bias of the interpreter. There are no embryological accounts of the following insect orders: Protura, Embioptera, Psocoptera, Thysanoptera, Strepsiptera, Aphaniptera, Anoplura and Mecoptera.

V. GENERAL DISCUSSION.

It is not easy to view the subject historically. Workers have taken up this aspect of embryology repeatedly and not infrequently arrived at different conclusions each time. It is difficult to decide whether these are changes of heart resting on prominent theories rather than concrete evidence, or real changes of opinion based on new observations. On the one hand, one is tempted to believe that in many cases the wish was father to the thought. In dealing with the developmental changes of minute cell structures in small embryos whose development is throughout obscured by the yolk, interpretation is too often a matter of personal bias. On the other hand, imperfect technique undoubtedly lead to much confusion. Much early theory rested on examination of whole mounts only, an obviously unsatisfactory method. The introduction of serial section technique improved the situation but here again the methods employed were rough. Hot water fixation followed by heroic methods of paraffin embedding must have set up sufficient convection currents in the embryos to alter the true relations of one layer to another and so lead to confounding results. Graber's account in 1890 should be specially mentioned, since he contributed to our knowledge of the embryology of a large number of insects and adequately reviewed the subject up to that time. In 1890 the theory of the mid-gut originating in the yolk cells seems to have been dispensed with and attention was focussed on (1) Kowalevski's view (1871) that the mid-gut arose as lateral strands separated from the mesoderm, (2) Grassi's (1884) and Heider's (1889) view that the mid-gut arose from the lower layer in anterior and posterior regions, (3) the views of Ganin (1874), Voeltzkow (1889), Witlaczil (1884) and Graber (1889), that the mid-gut rudiments originated from the blind ends of the stomodaeum and proctodaeum.

In 1889 Rabl in his "Theorie des Mesoderms," with an earnest desire to fit insect development to the germ layer theory, stated that when longitudinal invaginations occur in an insect embryo the median part undergoing invagination will form endoderm and the lateral, mesoderm. In this he wrongly interpreted Kowalevski (1871), and with the exception of Nusbaum (1888) the results of every worker up to that time were counter to Rabl's theory. Graber in 1890 brought a weight of evidence to bear on the subject in the form of his researches on *Musca*, *Bombyx*, *Hylotoma*, *Mantis*, *Pieris*, *Hydrophilus* and *Melolontha* and concluded in favour of the bipolar origin of the mesenteron. In 1895 Heymons published his classical work on the development of Orthoptera and Dermaptera. Devoting special attention to the mesenteron he found that it arose as ectodermal extensions of stomodaeum and proctodaeum. His work has had more influence than that of any other and has resulted in a crystallisation of the controversy into two widely opposed expressions of opinion. These views, depending primarily on whether the mesenteron is derived from ectoderm or true endoderm, may be regarded as of equal importance and merit. Subsequent workers find themselves accepting either one or the other view. From

what has been written it will be seen that the origin and nature of endoderm forms the corner stone to the main problem of insect gastrulation. The endoderm question in turn hangs closely with the origin of the mid-gut in insects and the origin, existence and significance of yolk cells. Following Leuzinger, Wiesmann and Lehmann (1926), we may consider these features separately, discussing first the views on the endoderm irrespective of its fate in organogeny and secondly the significance and origin of the mid-gut in insects.

(a) *The endoderm.*

The view of Heymons and his followers concerning the endoderm is that, as a germ layer formed by invagination, it does not exist in insects. The whole organogeny of the insect is effected at the expense of two layers only, namely ectoderm and mesoderm, and insects form a unique exception to the general rule in the coelomates in not possessing an endodermal germ layer, unless it be represented in yolk cells. Deegener (1900), Friederichs (1906), Hammerschmidt (1910), maintain that all three germ layers are present. The endoderm is formed by inward migration of cells from the blastoderm into the yolk (or the process may be shortened by cells remaining in the yolk during blastoderm formation). Such endoderm has only an embryonic significance and serves no purpose in organogeny except as a nutritional agent. The third view of which these are minor variations is that the lower layer produced by invagination or overgrowth gives rise to endoderm at its two ends and to mesoderm between them. Supporters of this view explain it in terms of separation of an originally continuous endoderm into anterior and posterior rudiments leaving the original lateral mesoderm in the middle line, Wheeler (1889, 1893), Kowalevsky (1871 and 1886), Bütschli (1888). A few observers maintain that the median strip of endoderm as a continuous tissue still exists, Hammerschmidt (1910), Mansour (1927), Eastham (1927), Nusbaum and Fulinski (1906), Hirschler (1909*b*), Philiptschenco (1912).

Another variant of this view is that endoderm arises at each end of the mesoderm band to form an anterior and posterior rudiment, but from its first appearance is independent of the mesoderm. Thus three definite and distinct germ layers are present. In support of this we have Grassi (1884), Carrière (1890), Escherich (1900), Noack (1901), Nelson (1915), Eastham (1927); Nusbaum and Fulinski (1909). A two-phased gastrulation is implied by the view of Deegener, Friederichs and Hammerschmidt. For if they regard the yolk cells as endoderm, gastrulation must begin even as early as the time when the blastoderm is being formed and only end when the mesoderm has been formed by invagination, overgrowth or multipolar immigration. Heymons had already considered this possibility but turned it down as a "luxury of nature." Actually there need be nothing remarkable in it; an analogous case is to be found in the gastrulation of the bird embryo where this process begins by delamination of hypoblast from epiblast, and is only completed with the formation of mesoderm at the primitive streak (Lillie, 1908). In support of this two-phased gastrulation, Dickel (1904), Noack (1901), have attempted to prove a genital relationship between yolk cells and definite endoderm by finding that the place of origin of yolk cells in the blastoderm is the same as the later place

of origin of endoderm cells. Unfortunately there are many cases where this does not hold. The work of Philiptschenko (1912) is of great importance since at last by his findings apterygote and pterygote embryonic development are brought into line with one another and he once and for all disposes of the idea that yolk cells take any part in organogeny. He also gives us an example of an embryo in which endoderm is continuous along the whole length consisting of anterior and posterior rudiments connected by a diffuse longitudinal layer passing from one end to the other. For him pterygotes only differ from apterygotes in the absence of the diffuse median portion. It is not surprising however to find this being retained in isolated pterygotes, as, for instance, in *Donacia* by Hirschler (1909b). This at once gives a tentative explanation of the bipolarity of the endoderm origin. Kowalevski (1886) recognised the division of the endoderm into anterior and posterior rudiments and offered an explanation of this which has been widely accepted. Owing to the great length of the insect embryo and the peculiarities of insect embryonic blastokinesis, endoderm has become restricted in its formation to the two poles and by this obviates any necessity on the part of the embryo to accommodate itself during blastokinesis to its changing position relative to the yolk. Such bipolar endoderm was originally continuous along the whole embryo. It is still retained in apterygotes but absent in the majority of pterygotes. Now if this theory has any value one might surely expect to find in a number of pterygote insects a vestige of the median endoderm. This has been found recently in Coleoptera and Lepidoptera by Mansour (1927) and Eastham (1927) respectively. In *Calandra*, Mansour finds a ventral invagination of the germ band and before this becomes enclosed as mesoderm those cells are passed from it into the yolk to assist in yolk liquefaction. In *Pieris*, Eastham finds a similar proliferation of cells from the germ band into the yolk before any mesodermal invagination takes place. For both these authors these cells represent a vestige of the median endoderm. Mansour however differs from Eastham in that he finds no bipolar endoderm rudiments. For him the mid-gut is ectodermal, the endoderm is purely embryonic and takes no part in organogeny. Apart from this latter difference of opinion their views on the median endoderm are interesting in that they tend to complete the story of Philiptschenko and fulfil the expectations expressed by Wheeler (1893) on Kowalevski's theory.

(b) Formation of the insect mid-gut.

In the light of these theories on the development of the endoderm we will now discuss the formation of the mid-gut. The development of the mesenteron epithelium obviously stands in close connection with germ layer formation. The oldest view of origin from yolk cells may be passed over with the briefest mention. It was a conclusion based on an ill-developed technique and many original adherents of this view abandoned it. Weismann (1863), Ganin (1869), Bütschli (1870) are associated with it. For them the mid-gut arose as a sac surrounding the yolk, enclosed anteriorly and posteriorly by the stomodaeum and proctodaeum, its wall being formed by free cell formation, Weismann (1863), or by cleavage, Rathke (1829).

The view of the origin of the mesenteron from yolk cells, Dohrn (1876), Mayer (1876), Gruber (1878 and 1889), Balfour (1880), Hertwig (1881), as a modification of the above resulted from the introduction of section cutting technique. With the introduction of the view of Grassi (1884) on bipolar origin of the mid-gut only few departed from the earlier view. Will (1888), Heymons (1897), Patten (1884) and Ayers (1884) still held to the yolk origin of mid-gut. For them apterygote development presented features very different from the most specialised pterygote development. Tschuproff (1903) attempted to show that Odonata occupied an intermediate position in that part of the mid-gut development in a bipolar manner apart from yolk. This result of Tschuproff has not yet been confirmed. Till Philiptschenko's work (1912) called in question all other investigators' results on apterygote mid-gut formation, the generally accepted conclusion was that only in these insects had the mid-gut a yolk cell origin. Then, Tschuproff's work had obviously an interesting significance, a significance which diminished in value at the appearance of Philiptschenko's work. If the Apterygota do not have a mid-gut originating in yolk cells, but one which develops in the manner of the gut in ordinary Pterygota, the Odonata on Tschuproff's results no longer occupy the intermediate position that was originally thought. The alternative interpretation of mid-gut formation is the bipolar view. It rests on the discovery of Grassi (1884) who proved that the mid-gut arose from two centres, anterior and posterior. By progressive growth of cells from these two centres in the form of paired longitudinal strands the mid-gut begins its development. Bütschli (1870) first suggested a bipolar origin of mid-gut, but afforded no proof of this. All authorities are now agreed that the two growth centres lie in the region of the mouth and anus respectively and as the stomodaeum and proctodaeum deepen these centres are carried deeper into the yolk. The difference of opinion seems to be purely an academic one. Are these centres of ectodermal origin arising from the blind ends of the stomodaeum and proctodaeum respectively, or do they arise independently of the stomodaeum and proctodaeum, and can they therefore be regarded as endoderm in the strict sense of the word? In examining the works of a number of authors in different insects I have come to the conclusion that in some cases the first appears to be certainly the case and in others the second appears equally true. If this is so, and there is no reason for supposing it to be not so, since the mid-gut of insects must be homologous throughout the insect orders, an attempt to bring the two views into agreement will not be out of place. The bipolar origin of the mid-gut independent of the stomodaeum and proctodaeum was regarded as correct by Grassi (1884), Kowalevski (1886), Heider (1889), Wheeler (1889), Escherich (1900), Schwangart (1904), Noack (1901), Nusbaum and Fulinski (1906 and 1909), Hirschler (1909), Strindberg (1914), Nelson (1915), Eastham (1927). At the time of the publication of Heymons' classical work on the Orthoptera and Dermaptera (1895) the view was kept temporarily in the background and revived by the authors who subsequently published work, mentioned above. Minor differences of interpretation are found in the works mentioned; for instance, the earlier workers did not recognise any distinction between mesoderm and the two rudiments giving rise to the mid-gut; in other words, the lower layer was for them a mes-entoderm.

In this category we find Grassi (1884), Heider (1889), Wheeler (1889), Hirschler (1909 b). Later workers, such as Nusbaum and Fulinski (1909), Nelson (1915), Noack (1901), Carrière and Bürger (1897), Eastham (1927), differ in that they find the two endoderm rudiments arising by a process quite distinct from that which produces mesoderm. They find, for instance, that mesoderm is formed in Hymenoptera, Diptera and Lepidoptera by the enclosure of the middle portion of the germ band *en masse*, the mid-gut rudiments arising from areas of cell proliferation. It is not surprising to find little distinction between mesoderm and endoderm formation in some forms since mesoderm itself can be produced in three different ways, *i.e.* by migration (*i.e.* proliferation), invagination or overgrowth and a combination of the three processes as occurs as in Lepidoptera, Eastham (1927), might easily obscure the distinct formation of entoderm. Another small difference of interpretation lies in the finding of Heider (1885) and Cholodkowsky (1891) where the mid-gut not only arises from the two centres but also from splanchnic mesoderm along the whole length of the embryo. The probability of this being an error has already been mentioned. Similar error in all probability lies in the conclusions of Hirschler (1909), Hammerschmidt (1910) on the partial origin of the mid-gut from the median mesoderm along the whole length of the embryo. These are however only minor "relapses" and leave the main point unaffected, *viz.*, that all can be in agreement as to bipolar non-ectodermal origin of the mid-gut, and for the most part with Wheeler (1893) give support to the interesting suggestion put forward by Kowalevsky (1886), (see p. 17 of this paper).

The view that the two rudiments forming the mid-gut are ectodermal in that they arise from the stomodaeum and proctodaeum respectively owes much of its vigour to Heymons (1895), though Witlaczil (1884), Voeltzkow (1889), Graber (1891) and others had already expressed the opinion with less certainty. After Heymons (1895), came Lecaillon (1898), Deegener (1900), Schwartz (1899), Toyama (1902), Tschuproff (1903), Czerski (1910), Hirschler (1905), Saling (1907), Friederichs (1906), Leuzinger, Wiesmann and Lehmann (1926), Mansour (1927). According to these authors, after mesoderm formation the stomodaeum and proctodaeum develop and by proliferation at their ends the mid-gut rudiments are formed. These authors belong to the group of workers who maintain that the true endoderm does not enter into the structure of the post-embryonic stages of insects. It is interesting to notice that, apart from the earliest workers in this category, the subjects for study have been members of the orders Lepidoptera, Coleoptera and Orthoptera, while the subjects for study by the authors in the former group who do not believe in an ectodermal mid-gut chiefly belong to the orders Lepidoptera, Diptera and Hymenoptera. An examination of the figures of these authors assures me of their correctness in the most important points. Are we to explain the difference of results in these orders in terms of actual significance of the mid-gut or in terms of academical interpretation? Is the mid-gut of the Coleoptera, for instance, no longer to be regarded as homologous with that of the Hymenoptera? If they are homologous, are we to discredit the authors describing the events in Coleoptera and credit those describing the Hymenoptera, or are we to accept the homology—accept

the findings of both and find the way towards reconciling the views? Since Heymons' time a sharp controversy has arisen over the whole subject for and against Heymons. In the attempt to prove one side right and the other side wrong the possibility that both might be right has been largely lost sight of.

In the short appendix to my paper on the embryology on *Pieris* (1927) I briefly suggested that there might not be as much difference between the two views on the origin of the mid-gut in insects as some authors supposed. There it was pointed out that Nelson (1915) in *Apis*, convincingly proved the presence of anterior and posterior mesenteron rudiments whose development had no connection with the ectodermal portions of the gut. Mansour in *Calandra*, just as certainly showed that the mesenteron developed from the blind ends of the stomodaeum and proctodaeum. There can be no question as to the homology of the mid-gut in these two insects and it was suggested that the real difference in development in these two embryos was one of degree and not of kind. The difference only concerns the time at which mesenteron rudiments are formed by proliferation relative to the time of formation of the stomodaeum and proctodaeum. In *Calandra* the rudiments only develop after stomodaean and proctodaean invaginations have begun, while in *Apis* and *Pieris* the same rudiments begin to appear before the stomodaeum and proctodaeum develop. An interesting point is that both Eastham (1927), and Mansour (1927), find a vestigial endoderm formed along the length of the embryos. Thus in *Pieris* it is found that vestigial endoderm arises by proliferation before any sign of mesoderm formation, but in *Calandra* the same cells are released *after* invagination to form mesoderm has taken place. The retarded development of the vestigial endoderm in *Calandra* as compared with the same development in *Pieris* is most significant and it is tempting to regard this as the clue to the whole situation. If endoderm is only formed after the longitudinal mesodermal invagination in *Calandra* it may surely be that the actual mesenteron rudiments—anterior and posterior—only form after stomodaean and proctodaean invaginations. In other words, the whole endoderm in *Calandra* is delayed in development and such delay gives the erroneous impression that it arises from the ectoderm. Another important point is that I have now evidence in *Pieris* that the anterior endodermal proliferation, which begins before the development of the stomodaeum, continues during stomodaean development, so that at this later time the mid-gut has the appearance of arising from ectoderm as was found by Toyama in *Bombyx* (1902). The fact that the proctodaeum develops almost synchronously with the posterior endoderm rudiments in *Pieris* only affords another instance of different time of appearance of mid-gut rudiments relative to the time of development of ectodermal mid-gut. There is therefore no great morphological difference in the tissue which gives rise to mid-gut in the two cases. After coming to the above conclusion I was glad to notice that, in 1909, Nusbaum and Fulinski had come to a similar one. They suggest that in forms hitherto regarded as having an ectodermal mid-gut, either the endoderm rudiments are formed late, or the stomodaeum and proctodaeum develop early so that the endoderm appears to have an ectodermal origin in the stomodaeum and proctodaeum. In support of this idea Nusbaum and Fulinski show that there are no less than seven different

types of development of mid gut rudiments in pterygote insects. These differ from one another in the precocity or lateness of development of the stomodaeum and proctodaeum compared with the mid-gut.

The types of development as tabulated by Nusbaum and Fulinski are as follows:

I. Anterior and posterior rudiments develop from the germ band before stomodaeum and proctodaeum, e.g. Noack (1901) in *Muscidae* and Hirschler (1909 b) in *Donacia* (Fig. 2 A).

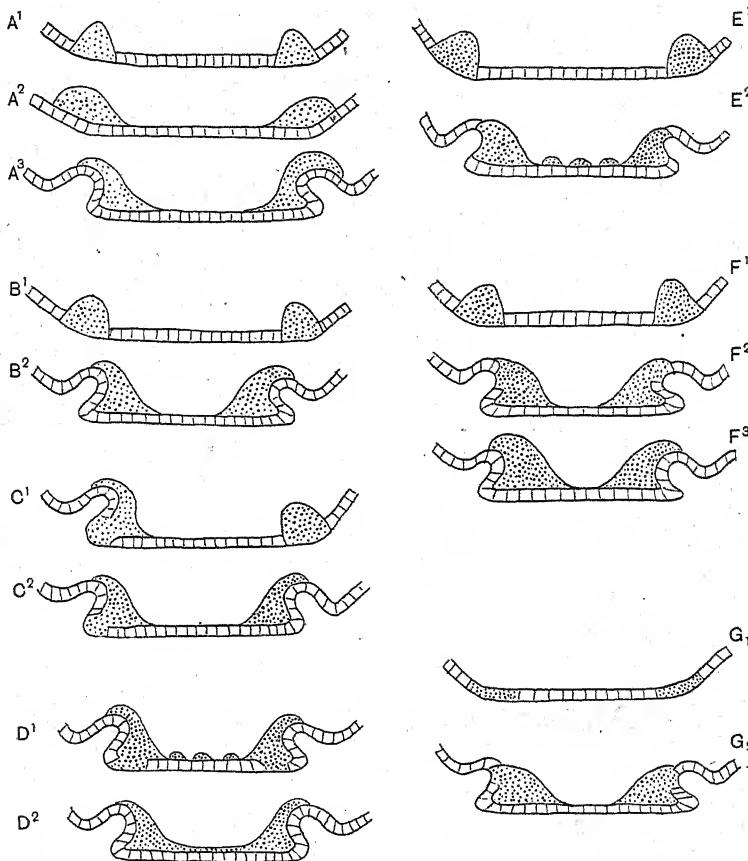


Fig. 2. Types of insect development after Nusbaum and Fulinski. Stippling represents endoderm—remainder ectoderm. For explanation see text.

II. This resembles type I except that stomodaeum and proctodaeum begin to develop before the mid-gut rudiments are completely severed from the germ band, e.g. Karawaiew in *Pyrrhocoris* (1893; Fig. 2 B).

III. Stomodaeum and anterior rudiments develop together, but the posterior rudiments precede the proctodaeum in development, e.g. Nusbaum and Fulinski (1909) in *Gryllotalpa* (Fig. 2 C).

IV. The stomodaeum and proctodaeum develop synchronously with the mid-

gut rudiments but not quite in the same positions, the anterior rudiments lying behind the stomodaeum and the posterior rudiments in front of the proctodaeum, e.g. Nusbaum and Fulinski in *Phyllodromia* (1906; Fig. 2 D).

V. Stomodaeum and proctodaeum develop before separation of the mid-gut rudiments from the germ bands. A median mesenteron rudiment also occurs, e.g. Hirschler (1909 a) in *Gasteroidea* (Fig. 2 E).

VI. Cell masses of the mid-gut rudiments occur. Then follows invagination of the stomodaeum and proctodaeum carrying the areas of the mid-gut rudiment formation inwards, e.g. Carrière and Bürger (1897) in *Chalicodoma* (Fig. 2 F).

VII. The area for mid-gut rudiment formation remains latent till the stomodaeum and the proctodaeum are well developed, e.g. Heymons (1895) in *Forficula* etc. (Fig. 2 G).

The above theory has this to commend it—it brings uniformity of interpretation where none existed before and it removes to a large extent that partisanship from a controversy which is apt to warp the mind of individual workers and lead to an unscientific bias in interpretation.

(c) *Nature of the so-called blastoporal groove.*

The discussion of this will of necessity be brief since it encroaches on what has already been written. The table on p. 26 clearly illustrates the main views on the whole subject and obviates the necessity for lengthy descriptions. The orthodox view is well expressed by Mansour (1927) when he says that "students of embryology recognise the gastrula as the stage produced by invagination of part of the blastula wall, the invaginated cells forming the lining of the primitive gut, this layer being endoderm." It is of course plain that any invagination which occurs in the insect germ band is more concerned with mesoderm than endoderm since the latter (if present at all) is only found anteriorly and posteriorly. The presence of endoderm and mesoderm together in these two regions might be sufficient reason for examining the embryo particularly here to see if there is any resemblance in their spatial relations and development to the development of these layers in other invertebrates. Kowalevski (1886), Bütschli (1888), Schmidt (1889), Noack (1901) examined this point in Muscid Diptera and found an almost ideal arrangement. In the most recent account of Noack (1901) we find that both anteriorly and posteriorly a condition obtains which strongly suggests the gastrulation of an alecithal egg. As seen in transverse sections, the embryo shows a deep invagination with a definite lumen projecting into the yolk. This invagination, which is continuous with that which passes along the whole germ band, is bounded at its innermost point by endoderm, and near the mouth of the opening at its junction with the ectoderm by mesoderm (Fig. 1 E).

The resemblance of the embryo in this condition to that of a generalised invertebrate embryo is most striking and led Bütschli (1888), Kowalevski (1886), Heider (1885), to the view that insect gastrulation only differed from the same process in other invertebrates by reason of the yolk and the length of the germ band. The longitudinal groove is for them a gastrular groove homologous with the same

structure in other invertebrates. The fact that the endoderm is not involved in the middle region of the embryo is again explained by them as being due to the length of the germ band causing a segregation of the endoderm to the two poles. If this view is right we might expect to find a more pronounced groove in generalised insects than in specialised orders, also a more distinct groove in embryos of less yolky eggs than of yolky eggs. That this is not so is seen by a review of the main insect orders.

In Collembola and Thysanura mesoderm formation is effected by multipolar immigration; in Orthoptera by delamination of cells from the edge of a pit or delamination of cells along the median part of the whole length of the germ band; in Lepidoptera, Coleoptera, Hymenoptera, and Diptera, by enclosure of the middle plate of the germ band by invagination or overgrowth. A parallel state of affairs is to be noted here to that found in endoderm formation, in that mesoderm develops at different times relative to the differentiation of the germ band. In *Anurida*, mesoderm develops before a definite germ band is formed, while in the higher insects not only is mesoderm formation postponed till after the formation of a germ band but till after it has undergone some slight differentiation. There might be said therefore to be in the insect series a progressive mustering of cell material from which germ layers are formed. The more specialised the insect the more complete is the preparatory period, *i.e.* more cell material is amassed before germ layers are formed. The less specialised the insect the less do we get a mustering of cell material, and germ layers are therefore formed immediately after cleavage. On these grounds it would seem difficult to homologise middle plate invagination with normal gastrulation of the annelidian type, *i.e.* blastopore type, since this is in all probability a new process evolved entirely independently within the class Insecta. This, of course, does not affect its being a process of gastrulation, and we might protest here against Mansour's definition of gastrulation (p. 22) and adopt that given by Brachet (1921): "Gastrulation is a process by which a two-layered larva is formed at the expense of cleavage products, the larva being so constituted that its two layers external and internal remain in continuity with one another at a determinate point. The gastrula consists in all cases of ectoblast and endoblast, *no matter how it comes about.*"

(d) *Yolk cells.*

In attempts made to determine whether the early stages of insect development were in agreement or at variance with the germ layer theory, the yolk cells occupy a position which varies with the view as to whether the mid-gut originates from the endoderm or ectoderm. The nuclei which lie in the yolk at the time when the primordia of the organs are laid down, have a dual origin. Some of the primary yolk cells have been present since blastoderm formation and represent those cleavage nuclei which remained in the yolk while the others wandered outwards to form the blastoderm; vide Bobretzky (1878), Grassi (1884), Patten (1884), Korotneff (1885) and in fact practically every worker on insect embryology. Others, called secondary yolk cells, result from an inward migration of nuclei from the germ band to the yolk at a later period. Though these are seldom distinguishable from the primary

cells, the fact that there are two categories must be noted. From the point of view of the germ layer theory the first or primary yolk cells alone are of importance here. It will at once be seen that if we accept the view of those workers who believe in the formation of true endoderm rudiments which grow into the mid-gut, we cannot regard the yolk cells as having any germ layer significance. Those investigators however who, with Heymons, maintain that the mid-gut arises from ectoderm, regard the yolk in different manners. Thus Heymons (1895) held that the insect embryo contained no true endoderm and that the yolk nuclei were a secondary feature. Lecaillon (1898), who likewise regarded the mid-gut as ectodermal, held that the yolk cells represented true endoderm which served only embryonic nutritional function with no significance in organogeny. Mansour (1927) regarded the yolk as a secondary feature, the true endoderm being still transitory but formed from cells which migrated inwards from the mesodermal (gastrular) furrow. All investigators into the Apterygota, though differing in the matter of the origin of the mid-gut, agree in the view that the yolk nuclei are a secondary adaptive feature.

Those embryologists who believe in a two-phased gastrulation process beginning at blastoderm formation and ending with the appearance of mesoderm, say that there are two developments of endoderm, one consisting of primary yolk cells formed at the same time as the blastoderm and being transitory and serving only a nutritive purpose, the other being definite endoderm which forms the mid-gut eventually, e.g. Hirschler (1909). The table on p. 26, modified from Mansour (1927), summarises the main views on the subject.

VI. OTHER ARTHROPODS.

The non-insectan classes of arthropods on the whole resemble the insects in possessing yolk eggs. The effect of this is the same in all cases, viz. to bring about the formation of a superficial blastoderm from which arises a germ band on the ventral side of the yolk. The differentiation of the germ layers differs in the several classes.

In the Crustacea, taking Manton's account (1928) as an example, an early differentiation of the germ layer anlagen occurs before gastrulation. A blastoporal area exists and from in front of this is formed the genital rudiment by inward migration. Ectodermal teloblasts are found at the anterior lip of the blastopore, and mesoderm is formed by inward migration of cells from behind the ectodermal teloblasts. Behind this region again certain cells pass in below the germ disc and absorb yolk. These form a yolk sac and by differentiation first at the stomodaeum and proctodaeum the definitive mid-gut epithelium is formed. The difference between this mode of development and that of the insect is very marked. Similarity does exist, however, in that first a germ band is formed and, from this, cells pass in to form the germ layers (cf. Wheeler's account of *Phyllodromia*, 1899). The formation of endodermal end-plates against the stomodaeum and proctodaeum might be comparable to the formation of endodermal rudiments in insects, and the early absorption of yolk by the cells which go to form such end-plates leads one to the speculation that perhaps insect yolk cells and endodermal rudiments represent

one germ layer, some elements of which, the yolk cells, have lost their power to build an organ in their precocious function as yolk absorbers. MacBride (1915) describes the gastrulation process in *Astacus fluviatilis* after Reichenbach (1888) and of *Homarus* and *Palaeomon* and these agree broadly with Manton's account. In the case of the *Arachnida*, Kishinouye (1891 and 1893) gives us accounts of the process of germ layer formation in the spider *Agelena* and the Xiphosuran *Limulus* respectively, see MacBride (1915). From the primary ventral thickening of the embryo of *Agelena* an inner keel of endoderm is formed. Cells from this spread out and surround the yolk and ultimately form the mid-gut. The rest of the keel flattens out into two bands to form mesoderm. In *Limulus* the development is similar except that mesoderm cells are budded into the yolk before the formation of an inner keel. When the latter appears it therefore consists of mesoderm. In *Agelena* we may therefore speak of endoderm being formed from a lower layer of mes-endoderm, compare many insects, while in the Xiphosura a separation of the two germ layers is effected by the precocious development of endoderm. The inward passing of this tissue to the yolk strongly recalls the same process of transient endoderm formation noted by Mansour (1927) in *Calandra* and by Eastham (1927) in *Pieris*. The incomplete account of the Pantopodan development given us by Morgan (1891) presents no notable features different from the above. Erlanger (1895) finds that in the Tardigrada the egg is deficient in yolk. Cleavage is holoblastic, a gastrula is formed by invagination and the coelom arises as archenteric diverticulae. The development here is so atypical for the Arthropoda as to be difficult to comment upon. The Myriapoda (see Heymons, 1901) have a development which is strikingly similar to that of insects. The blastoderm is formed as in insects and from a thickening in this, cells are budded which form a mid-gut epithelium. The cells forming this are indistinguishable from yolk cells. From the hinder end of the thickened plate, mesoderm is formed which extends as far forwards as the definitive embryonic area. Here again we find evidence for the suggestion that yolk cells and endoderm have a common phyletic origin. This is no reason however for assuming (Mansour, 1927) that the whole of the insectan endoderm is purely embryonic and plays no other part in organogeny than a nutritional one. The differentiation of yolk cells in Hemimysis (Manton, 1928) to form the actual mid-gut, first in the region of the stomodaeum and proctodaeum, might just as reasonably be taken as tentative evidence in favour of the view that yolk cells (endoderm) in insects have become divided into two categories, one of which (the vitellophags) is now purely embryonic and the other (endoderm rudiments) is organogenic.

Table I.

Stage or layer	Kowalevski	Lecaillon	Hirschler	Mansour	Eastham	General view on Aptygota	Philip-tschenko
Blastoderm stage	Blastula	Post gastrula	Post gastrula	Blastula	Blastula	Blastula	Blastula
Primary yolk cells	Secondary feature	True endoderm	Primary endoderm	Secondary feature	Endoderm	Secondary feature	Secondary feature
Ventral groove	Gastrular furrow	Secondary feature	Secondary feature	Gastular furrow	Secondary feature	Absent, gastrulation multipolar	Absent, gastrulation multipolar
Walls of groove	Meso- and endodermal	Mesoderm	Meso- and secondary endoderm	Meso- and (transitory) endoderm	Mesoderm	—	—
Cells budded into yolk	—	—	Secondary feature	Endoderm	Endoderm	Endoderm	—
Inner layer after loss of cells to yolk	Mesoderm and anterior and posterior endoderm	Mesoderm	Mesoderm and anterior and posterior endoderm	Mesoderm	Mesoderm and anterior and posterior endoderm	Mesoderm	Mesoderm and endoderm rudiments

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TEMPERATURE COEFFICIENTS IN BIOLOGY

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(Received August 21, 1929.)

(With Seven Text-figures.)

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I. INTRODUCTION.

THE biological problem of temperature coefficients suffered from the beginning from the fatal circumstance that it was not initiated by biologists, but by chemists. It has of course long been known both to plant and to animal physiologists that biological processes are accelerated by raising the temperature up to the optimum, but for a long time no attempt was made by biologists to study this common phenomenon on a more quantitative basis and to draw general conclusions as to its nature and meaning.

It was undoubtedly the merit of some chemists, chiefly of Van't Hoff, Cohen, Abegg, Herzog, Arrhenius, that they drew the attention of biologists to the possibility of a more quantitative and deeper study of the action of temperature on living organisms. At the same time, however, this purely biological problem has been misled by a chemical line of thought into a blind alley.

The beginning of this biological problem may be found in chemical literature. In the Van't Hoff's lectures on physical chemistry (Van't Hoff and Cohen (1896)), Clausen's (1890) experiments on the respiration of plants are discussed and the temperature coefficient Q_{10} is stated to be equal to 2.5. Another mention of the Q_{10}

of biological processes is to be found a few years later in Cohen's (1901) book, where among other data, those of O. Hertwig on the tadpole development are recalculated and values of Q_{10} given. O. Hertwig (1898) himself mentioned Van't Hoff's rule with some hesitation and emphasised that the acceleration of development in the frog by temperature cannot be reduced to the mere acceleration of biochemical processes inside the egg. Yet further advances in the question were made by chemists. In 1903 Aberson calculated the Q_{10} of the velocity of alcoholic fermentation and found it to lie between 2 and 3. In the 1905 volume of the *Zeitschrift für Elektrochemie* four papers by Abegg, Cohen, and Herzog were published, all dealing with the Q_{10} of biological processes.

The first biologist who took over the doctrine of Q_{10} from the chemists and who believed in it was Kanitz, whose first paper on the subject (1905), primarily destined for a botanical periodical, appeared in connection with the previously mentioned chemical notes. After him and partly simultaneously with him, some other biologists began to deal with the problem. Blackman (1905), Peter (1905), J. Loeb (1905), Snyder (1905) were among the first, and in the following years the number of papers dealing with the question rapidly increased.

In spite of this widespread interest of biologists, the positive results of a more general character are rather poor, as we shall see later, and the problem of temperature action in biology still remains an open question. Of late years, Crozier and his collaborators have tried to develop the question, but it cannot be said that their work has brought a solution.

The relative failure of this extensive investigation may be explained by the fact that many biologists, misled by purely chemical ideas, have forgotten the essential feature of living matter, namely its heterogeneous character. It is not without interest to note that not only O. Hertwig (1898), but even Cohen (1901) laid stress on the probable complexity of biological processes, but biologists have readily neglected this important feature of living matter.

It is the chief purpose of this review to collect data showing that the reaction velocity in heterogeneous systems, and thus in the protoplasm, obeys laws which are different from those established for reactions in homogeneous systems, and that the velocity of biological processes depends on the rate of diffusion in viscous media.

II. REVIEW OF TEMPERATURE FORMULAE.

(a) Berthelot's exponential formula and the Q_{10} rule.

The rule of Q_{10} , commonly used in biology, may be derived from the temperature formula, which was proposed by Berthelot (1862) as a first approximation for some chemical reactions:

$$K_1 = A \cdot B^{t_1} \quad \dots \dots (1),$$

where K_1 is the reaction velocity at the temperature t_1 , A and B constants of the equation. According to this empirical formula, the reaction velocity is an exponential function of temperature. The same formula may be also written:

$$\log K_1 = \log A + t_1 \cdot \log B \quad \dots \dots (2),$$

and when we put $\log A = a$, $\log B = b$, the equation becomes:

$$\log K_1 = a + bt_1 \quad \dots\dots(3),$$

which means that the logarithm of the velocity is a linear function of the temperature. In other terms, the Berthelot's formula supposes that the velocity increases in a geometrical progression, when the temperature rises arithmetically. But it soon became evident that this formula no longer holds good when the range of temperatures is large enough. Van't Hoff (1884) has therefore proposed a new formula, generally now called the Arrhenius or Van't Hoff-Arrhenius law and with which we shall deal later. By a simplification, allowed for temperatures not too distant from each other, this law takes the form of the Berthelot's equation (1), (3). Thus simplified the Van't Hoff's law may be expressed as follows: when the temperature rises by 10° C. the velocity of most chemical processes increases twice to three times. Hence the relation:

$$\frac{K_{t+10}}{K_t} = Q_{10} = 2 \text{ to } 3 \quad \dots\dots(4),$$

where K is the velocity constant at temperatures t and $t + 10^\circ$ respectively, and the quotient Q_{10} a constant, generally called the temperature coefficient of Van't Hoff. It is obvious that the value of Q_{10} is in a close relation to the value of the constant B in the Berthelot's formula (1), (2). This relation is (see Kanitz (1915)):

$$\log Q_{10} = 10 B \quad \dots\dots(5).$$

Van't Hoff and Cohen (1896) have emphasised that this rule holds good not only for many chemical reactions, but for biological processes as well. The same rule is often designed by German authors as "RGT-Regel" (Reaktionsgeschwindigkeit-Temperatur-Regel), according to Kanitz (1915). As a matter of fact, it has been demonstrated by many investigators, who have made the question a scientific study and whose work has been summarised by Pütter (1914), Kanitz (1915), Matisse (1919), H. Przibram (1923), Kanitz (1925) and Pütter (1927), that most of the biological reactions give a Q_{10} lying between 2 and 3. However at the same time it has been shown (a) that many biological processes give values much lower or much higher than this, and (b) that the value of Q_{10} for one and the same reaction is not constant, but that it varies with temperature (Kanitz (1907 b)).

As to the cases, in which the Q_{10} is lower than 2 or higher than 3, some authors have pointed out that many processes of physical character give Q_{10} lower than 2, and have therefore thought that it would be possible to classify biological processes into "physical" and "chemical" ones. We shall see later that this attempt has not been successful.

As to the inconstancy of the Q_{10} at different temperatures, it has first been attributed to imperfections of experimental technique. Therefore Snyder (1907), Loeb (1908) and others first simply took the average of all values observed and found that the average Q_{10} thus obtained usually lay between 2 and 3. Yet it soon became obvious that the inconstancy of Q_{10} with temperature was not accidental, but was systematic, and that at lower temperatures the values of Q_{10} generally are high, whereas at higher temperatures they are low. Fauré-Fremiet (1925) gives a

graph showing variations of Q_{10} with temperature and demonstrating that they are continuous and regular.

Kanitz (1905) and Snyder (1911) have pointed out that even in chemical reactions the Q_{10} varies with the temperature, and Snyder (1911) gave some examples thereof. From the data, found by Harcourt and Esson (1895) for the dissociation of hydrogen peroxide with iodine hydrogen, the following values of Q_{10} may be calculated:

Temperatures	Q_{10}
0-10	2.080
10-20	2.078
20-30	1.940
30-40	1.932
40-50	1.912

It is evident that even in this purely chemical process the Q_{10} is not constant, yet it depends on the temperature to a much lower degree than is usually the case in biological phenomena. Van't Hoff (quoted from Snyder (1911)) himself knew this to occur in chemical reactions and attributed the phenomenon to the viscosity of the medium. Plotnikow (see Kanitz (1907 a)) has found that even in chemical reactions the Q_{10} may exceed the value of 3 at very low temperatures and that it falls below 2 at high temperatures. These variations however only become important when extreme temperatures are concerned. On the contrary, the same or greater variations of Q_{10} occur in biological processes at temperatures lying quite close to one another.

The inconstancy of Q_{10} at various temperatures may be explained in four different ways:

(1) Tammann (1895) has shown that the velocity of enzymatic processes first increases with increasing temperature and then decreases when the optimum has been reached, and he has ascribed this phenomenon to the destruction of the enzyme at high temperatures. Duclaux (1898-9) somewhat later adopted the same point of view and tried to treat the subject in a more quantitative way. Blackman (1905) applied this principle to living matter and explained the variations of Q_{10} with temperature by the assumption that the activity of the living substance is slowly destroyed at temperatures approaching the optimum. As a matter of fact it is derived from the experiments of Matthaei (1905), that the velocity of plant assimilation steadily decreases with time at temperatures lying close to the optimum. Blackman's explanation, however, was criticised by Kanitz (1915). The chief objection made by this author is that a decrease of activity with time could only be experimentally established at $+25^{\circ}$ and higher, while the Q_{10} decreases in value from the lowest temperature (-5°) onwards.

(2) Kanitz's (1915) explanation is that the Q_{10} must necessarily deviate at and near temperatures which are the limits of life and which act differently from those which are "normal" or "biokinetic" (Kanitz (1925)). This author suggested that the starting of enzymatic activity at low temperatures is connected with a high Q_{10} , as is also its destruction by high temperatures. But this explanation is not convincing, either because low and high temperatures arrest the enzyme action by

quite a different mechanism, the arrest being reversible in the first case and irreversible in the second. Secondly, the variations of Q_{10} with temperature are evident not only at and near the limiting temperatures, but at all other temperatures as well.

(3) The inconstancy of Q_{10} at different temperatures has led some biologists to assume that every biological process is based upon a catenary series of chemical and physical processes, each of which has its own temperature coefficient and becomes the "limiting factor" (Blackman (1905)) or "master process" of the whole at a definite temperature. This explanation, known as the Blackman-Püttner principle (Püttner (1914)), has not been demonstrated in reality. On the contrary, the progressive and regular decrease of Q_{10} with increasing temperatures makes such an explanation doubtful.

(4) The fourth explanation of the inconstancy of Q_{10} at different temperatures is that the rule of Van't Hoff does not hold good in biological reactions. So many investigators came to this conclusion, that we can only mention the names of some of them: Filon (1911), Krogh (1914 a, b), Matisse (1921), Burns (1921), Dirken (1922), Heilbronn (1922), Pantin (1924), Fauré-Fremiet (1924, 1925), Heller (1925), Mangold (1926), Potonié (1926), Buccianti (1927), Ludwig (1928), Andersen (1929).

In many biological reactions the Q_{10} decreases to such an extent with increasing temperature, that it varies to about the same degree as the temperature is varied. The following table, borrowed from a paper of Dirken (1922) on the rate of metabolism in the cockroach, *Periplaneta americana*, clearly shows this:

Temperatures	Q_{10}
10-20	4.31
15-25	3.39
20-30	1.78

But the value of Q_{10} often varies even considerably more. From the data recently published by Ludwig (1928) on the action of temperature upon the development of an insect, *Popillia japonica* Newman, it is easy to calculate that for the narrow range of temperatures between 15° and 30° , the Q_{10} of the rate of egg development decreases more than fifteen times, when the temperature is raised only twice. W. M. Bayliss (1924) remarks that a temperature constant, which varies with one of the variables, is no true constant. Biological literature furnishes much evidence for this statement. The Q_{10} could have some meaning as a comparative quantity only when determined for two very near temperatures, which of course had to be chosen the same in every case. But even with this restriction, a serious objection remains, because one and the same temperature may have a different biological meaning for two different organs or organisms. The temperature of $+25^{\circ}$, which is the optimum for many plants and cold-blooded animals, is too low for the activity of brain centres in mammals (see Lefèvre (1911)). A temperature at which one organ or organism starts its activity may therefore be too high and injurious to another. With these difficulties a serious analysis by means of the Q_{10} is not possible, and the practical use of this constant for comparative study will not therefore be very significant. It may be explained only by the well-known mathematical timidity

of biologists, that the Q_{10} still figures with some importance in many biological publications.

(b) *Corrected exponential formulae.*

Kafka, jr. (1921) records that Stephens and Barrow found the following formula for the action of temperature upon the development of *Drosophila melanogaster*:

$$y = A^x - Bx^n \quad \dots\dots(6),$$

where y is the velocity, x the temperature, A the temperature coefficient, B and n another two constants. This formula, which may be derived from the simple exponential, has not been demonstrated in any other case except just in the development of *Drosophila*.

Another correction of the exponential temperature formula has been proposed by Janisch (1925, 1927). This author takes up the principle of Tammann, Duclaux and Blackman, according to which the decrease of Q_{10} with rising temperature is explained by a partial destruction of enzymes or living matter at higher temperatures, and gives a quantitative expression thereof in the form of the following equation:

$$y = (m/2)(a^x + a^{-x}) \quad \dots\dots(7),$$

where y is the time necessary for the given reaction, m the time at optimum temperature, x the temperature in degrees Centigrade, and a a constant.

The graphical representation of this equation is a curve with two slopes, connected at the optimum. Its external appearance is much the same as that of the biological temperature curves. Yet this formula has been subjected to some criticisms by Dingler (1926), Martini (1928), and the writer (Bělehrádek (1928)). The chief objections made by these authors are: (a) the formula has been demonstrated to hold good only in a single case, namely the development of the moth *Ephestia kühniella*, and even here the number of observed points is too low to allow any general conclusion. For the temperatures above the optimum, the curve has not been convincingly demonstrated at all, so that one of the two slopes of the curve is entirely in the air. (b) The formula has so many constants, that when these are appropriately chosen, the equation will hold good in many cases. The same cases however could be expressed as well by any other formula, which would be similarly rich in mathematical symbols (Martini (1928)). Apart from these formal deficiencies, Janisch's idea suffers from certain biological errors. The author seems to neglect the fact that the velocity at temperatures above the optimum is not constant, but that it varies considerably with time, so that the longer the time of exposure to a definite temperature, the lower will be the velocity. The constants of Janisch's formula would therefore necessarily vary with the time of exposure. In other words, quite a different set of processes take place in the cell at temperatures above the optimum from those which occur at "normal" temperatures, and there is no advantage in considering these two different sets of processes from the same point of view nor in mingling them in a single quantitative law. Janisch further supposes that the protoplasmic destruction occurs even at the lowest temperatures, which has not been demonstrated.

In his more recent publication, the same author (Janisch (1927)) has attempted to show that exponential relations of the most varied mathematical forms are characteristic of biological phenomena in general and that the exponential temperature formula is only a special case of this "exponential law in biology." This point of view, however, exposes itself to a serious criticism both from the mathematical and the biological sides (see Boresch (1928), Bělehrádek (1929)). It actually conceals in itself the danger that the mathematical treatment of biological phenomena might be turned into a mere mathematical play.

(c) *The rule of thermal summation and Krogh's linear relation.*

The oldest attempt at a quantitative analysis of the temperature action in biology was the rule of thermal summation, emphasised as early as 1851 by Boussingault and others. This rule assumes that the time (y), necessary to reach a definite stage of development, multiplied by the temperature (x), gives a constant:

$$x \cdot y = K \quad \dots\dots(8).$$

This rule has been shown to hold good not only in many cases of plant development (Boussingault (1851)), but also in the development of insects (see especially Hennings (1907), Sanderson (1908, 1910), Blunck (1924), Bodine (1925) and Ludwig (1928)). The temperature (x) is to be counted from the "biological zero," which is the temperature at which the development is stopped by cold, and not from the physical 0°C . (Dingler (1926)).

Krogh (1914) emphasised that the relation between the temperature (x) and the velocity (v) of many biological processes is linear:

$$v = k \cdot x \quad \dots\dots(9).$$

Because the velocity may be taken as the reciprocal of time ($v = 1/y$), the same relation may be also written:

$$1/y = k \cdot x \quad \dots\dots(10),$$

and when one puts $k = 1/K$, the formula becomes:

$$x \cdot y = K \quad \dots\dots(11),$$

which is identical with the formula (8). In other words, the linear relation of Krogh, found also by Knowlton and Starling (1912) for mammalian heart-beat and recently recorded in more numerous cases by Pütter (1927), is identical with the rule of thermal summation.

We shall see later that this rule and the Krogh's linear relation are but special cases of a more general relation and that they are nearer to the reality than any other temperature formula yet proposed.

(d) *Van't Hoff-Arrhenius law.*

We have seen that the Berthelot's exponential formula for chemical reactions gives a temperature constant which varies with temperature. Van't Hoff (1884) has therefore proposed a more exact formula, which is:

$$K_1 = K_2 \cdot e^{\frac{\mu}{T_2} \left(\frac{T_2 - T_1}{T_1 T_2} \right)} \quad \dots\dots(12).$$

In this equation, K_1 and K_2 are velocities at temperatures T_1 and T_2 respectively, the temperature being counted from the absolute zero. The constant μ , generally called temperature characteristic or thermal increment, indicates the amount of heat involved in the reaction (see Feldman (1923)) and is practically independent of the temperature, as far as simple chemical reactions are concerned. This formula is now generally used in chemistry (Matisse (1921)), although it does not always entirely agree with the observed facts. It supposes that the reaction velocity becomes zero at -273° , but many chemical reactions are known to be stopped at temperatures much above the absolute temperature zero (see Boutaric (1927)). Arrhenius has elaborated the theoretical basis of the formula, which therefore often bears the name of this physicist (Kanitz (1915)). Arrhenius (1907, 1908) was also the first who tried to apply the formula to certain biochemical processes. He pointed out that the destruction of some organic substances by heat gives a very high value of μ , while other biochemical processes are influenced by temperature to about the same extent as are ordinary chemical reactions. Among the biologists, Snyder (1907, 1908) and Rogers (1911) used the formula for certain physiological processes. The majority of biologists, however, contented themselves with the Q_{10} rule and it is only quite recently that Matisse (1919, 1921), Robertson (1920), Hecht (1926, 1928), Brown (1926, 1928) and Crozier with his collaborators (Crozier (1924 a, b, c, 1926 a, b, c), Crozier and Federighi (1924 a, b), Crozier and Stier (1925, 1926 a, b, 1927 a, b) have tested the formula in biology once more. Crozier has devoted much work to prove that the formula holds good in biology and to elaborate a hypothesis, with which we shall deal later. Here we shall consider whether or not the experimental data adhere to the Van't Hoff-Arrhenius formula.

Rogers (1911) has noticed that the value of μ for the heart beat in some lower animals is not constant, but that it varies with temperature. Many authors after him have made the same statement. This inconstancy of μ in biological processes may easily be understood when we consider that there exists a close mathematical relation between Q_{10} and μ . The value of μ varies with temperature in a similar way and for the same reasons as does the value of Q_{10} . Let us take a few examples to show how important these variations are:

(1) Larval development of *Drosophila* (Bliss (1926)). -

Temperatures	μ
12-16	33,210
16-25	16,850
25-30	7,100

(2) Rhythm of the contractile vacuole in *Paramecium* (Cole (1925)).

Temperatures	μ
< 16	25,600
16-22	18,600
22-31	8,600

(3) Duration of the fifth instar in *Bucculatrix canadensisella* (Friend (1927)).

Temperatures	μ
12-15	20,644
15-21	16,637
21-25	14,709
25-29	9,694

Similarly, as already demonstrated for the variations of Q_{10} with temperature, the value of μ often varies more than the temperature itself. From the experimental results of Ludwig (1928), mentioned above, it is evident that μ diminishes six times, when the temperature is raised only twice. Variations of μ with temperature have been the starting point of criticisms recently offered by some authors as to the adequacy of the Van't Hoff-Arrhenius formula in biology (Heilbrunn (1925), Bělehrádek (1928 b), Ludwig (1928), Fulmer and Buchanan (1929), Bělehrádek (1929)). As already stated above, the reaction velocity ought to become zero, conformly to the Van't Hoff-Arrhenius law, at the absolute temperature zero. For biological reactions, however, this never occurs, because they invariably are stopped at much higher temperatures, lying near 0°C . Many biological processes are even known to be arrested at temperatures much above 0°C . Now when we consider that the range of biological (biokinetic, Kanitz (1925)) temperatures comprises less than 40°C ., the difference of about 273°C ., which separates the biological zero from the absolute temperature zero and which is neglected when the Van't Hoff-Arrhenius formula is used in biology, is about seven times as great as the whole biokinetic temperature range itself.

The simplest way to test the formula is to plot logarithms of observed velocities against reciprocals of absolute temperatures (Crozier, 1924 a, b, etc.). But the range of biokinetic temperatures being rather narrow, the reciprocals of $273^{\circ} - 313^{\circ}$ abs. T° ($= 0^{\circ} - 40^{\circ}\text{C}$.) are practically linear function of the ordinary temperature (see graph in Bělehrádek (1928 b)). In other words, there is no practical difference between the Q_{10} rule and the Van't Hoff-Arrhenius law in biology. Therefore biologists cannot expect any real advantage in using the Van't Hoff-Arrhenius formula, because it guarantees about the same very low degree of accuracy as the Q_{10} rule, but is more complex than the latter.

But even among the enzymatic processes, many have been shown not to follow the Van't Hoff-Arrhenius law. Ernström (1922) found the following variations of Q_{10} and of μ for the activity of an amylase:

Temperatures	Q_{10}	μ
0-10	3.2	17,900
10-20	2.2	12,800
20-30	2.0	12,100
30-37	1.4	8,600
37-40	1.1	7,200

Fig. 1 is a graphical representation of the relationship between Q_{10} and μ in this special instance, but similar curves might be drawn for any case where the formula proves unsatisfactory. Whenever Q_{10} decreases with increasing temperature, the value of μ necessarily follows the same course and *vice versa*. This has also been demonstrated by Robertson (1920).

The example just cited points to another important conclusion, namely that neither the Q_{10} rule, nor the Van't Hoff-Arrhenius formula holds good for chemical processes in heterogeneous systems. Similarly Euler and Laurin (1920) found that the inversion of cane sugar by means of hydrochloric acid gives $\mu = 25,600$, constant for a large zone of temperatures, while the inversion with the aid of a sucrase is

accompanied by a μ which is no longer constant, but steadily decreases with increasing temperature from 11,400 to 5800.

One cannot expect that a chemical law, which does not hold good in enzymatic processes, should yield better results in processes taking place inside the living cells. It may be said that the Van't Hoff-Arrhenius law has no real validity in biology.

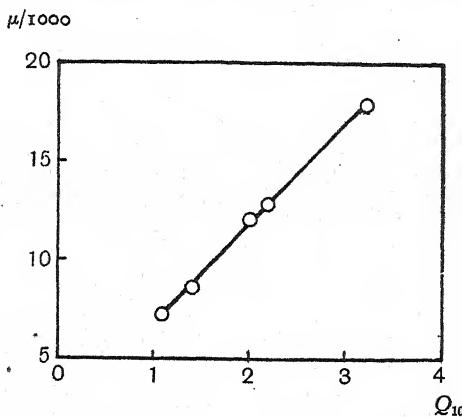


Fig. 1. Temperature characteristic μ and temperature coefficient Q_{10} are in a close mathematical relationship. (Action of temperature on the reaction velocity of an amylase, according to Ernström.)

(e) New empirical formula.

Porodko (1926 a, b) demonstrated that the relation between the intensity (x) of a stimulus and the time (y), necessary to provoke a reaction in plants, may be expressed by the equation:

$$y = k/x^m \quad \dots\dots(13)$$

where k and m are constants. The same formula may also be used, according to this author (Porodko (1926 b)), for the heat coagulation of proteins and for the killing of protoplasm by high temperatures. The exponent m was found by Porodko to lie between 8.8 and 44.0.

At about the same time and independently from Porodko, the writer (Bělehrádek (1926 a, b)) showed that the acceleration of the majority of biological processes by temperature follows a general rule, expressed by the formula

$$y = a/x^b \quad \dots\dots(14)$$

in which x is the temperature in degrees Centigrade, y the time necessary to accomplish a reaction, or the reciprocal of velocity, a and b constants. The exponent b is a temperature coefficient. It is evident that the formula is mathematically identical with Porodko's equation (13), though each of them concerns a different biological phenomenon. This is probably due to the current practice used in getting equations for empirical curves which consists in successively putting into logarithms first the axis x alone, then the axis y alone, or both the axes x and y at the same time (see Feldman (1923)). The formula given above (13, 14), as well as

that proposed by Wo. Ostwald (1907) for the toxic action of certain substances in various concentrations, are transformed into equations of a straight line when both axes are logarithmic:

$$\log y = \log a - b \cdot \log x \quad \dots\dots(15).$$

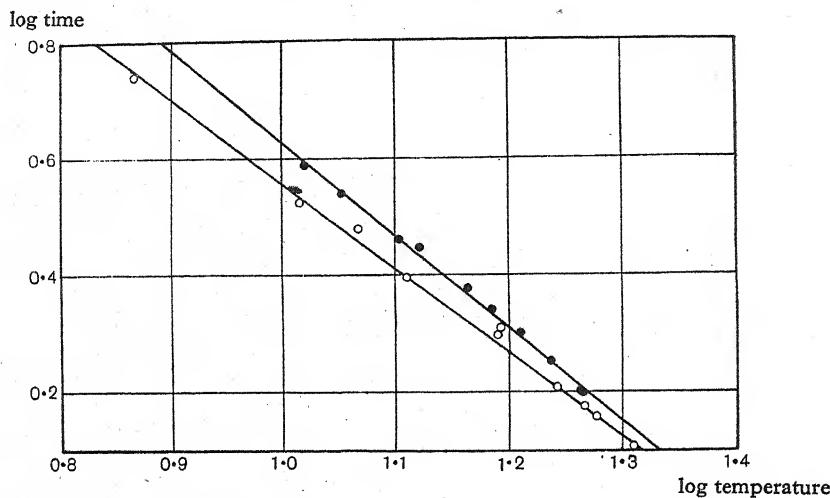


Fig. 2. Action of temperature on the time of reaction in the photosensory process in *Ciona* (white circles) and in *Pholas dactylus* (black circles) (Hecht (1926, 1928)).

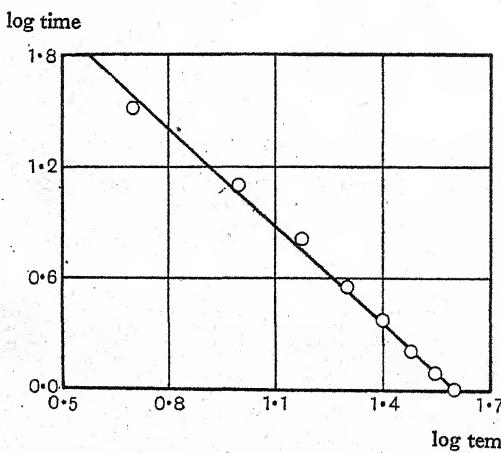


Fig. 3. Action of temperature on the relative velocity of alcoholic fermentation in *Saccharomyces cerevisiae*, cultivated on levulose (Slator (1908)).

This also is the simplest way for testing whether the formula (14) holds good in a given case or not. Logarithms of observed times or velocities, plotted against logarithms of temperatures, are the better grouped on a straight line, the more accurately the observed data adhere to the formula.

Figs. 2-5 demonstrate the validity of the formula in certain instances. Further

examples may be found in the previous publications of the writer (Bělehrádek (1926 *b, c, d*, 1929)).

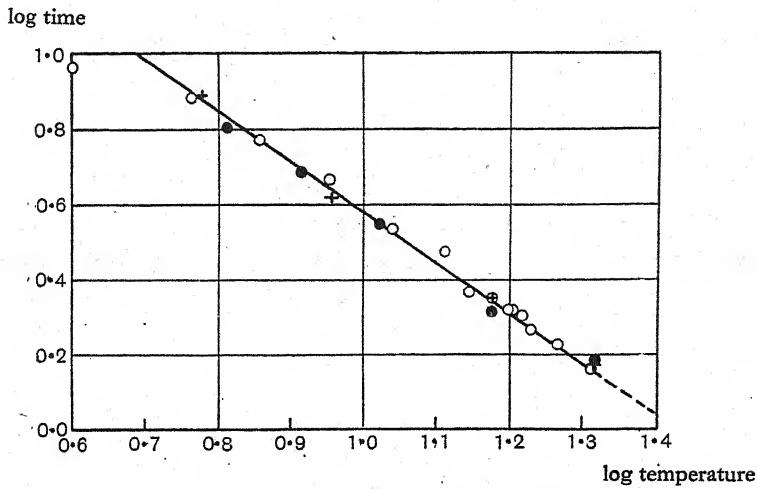


Fig. 4. Action of temperature on the refractory period in the frog's nerve, according to Adrian (1916).

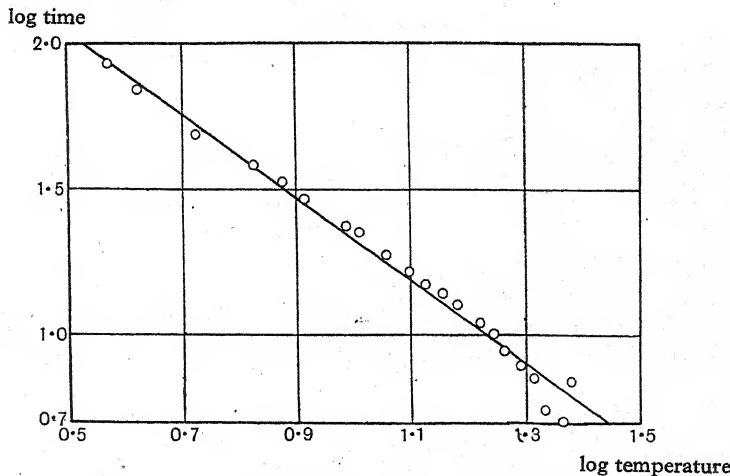


Fig. 5. Action of temperature on the velocity of oxygen consumption in *Astacus fluviatilis*, according to Brunow (1911).

When two points are known, the coefficient b may be obtained by means of the equation:

$$b = \frac{\log y_1 - \log y_2}{\log x_2 - \log x_1} \quad \dots\dots(16),$$

or, when velocities are used instead of times:

$$b = \frac{\log v_2 - \log v_1}{\log x_2 - \log x_1} \quad \dots\dots(17),$$

When the temperatures (x_1, x_2) are chosen such that the difference of their logarithms is equal to 1 (for instance 2° and 20° , or 3° and 30°), the difference of the logarithms of corresponding times (velocities) gives b directly.

Crozier and Stier (1927 a) state that the formula¹ does not agree with Fries' (1926) experimental data on the heart frequency of the cockroach. But firstly, it is not probable that the formula could be applied in every case with the same accuracy, and secondly, the experimental technique of Fries suffers from serious deficiencies (see Bělehrádek (1929)). On the contrary, Artom (1928), who has tested the formula for the velocity of nervous conduction, finds it sufficiently accurate.

As to the practical use of the formula, some remarks are necessary.

The formula being empirical and thus only a first approximation, one cannot expect it to hold good in various instances with the same degree of accuracy. Nevertheless, it agrees fairly well with many experimental data for a large zone of temperatures. This is especially true for processes in which the Q_{10} and the μ systematically vary with temperature, as may be seen from some examples cited in a previous paper (Bělehrádek (1929)). In some other instances, the formula seems to be less accurate, but it often happens that for one and the same reaction, it agrees with the observed data quite well in one organism and rather badly in another. This is very often due to technical sources of error chiefly at low temperatures, where it is not enough to wait a quarter of an hour for the temperature equilibrium, as is the common practice in biological experiments, but sometimes several hours, before the velocity becomes constant (Bělehrádek (1929)). The author has shown that very small organisms, in which the temperature equilibrium, controlled thermoelectrically, is reached in a few minutes, attain, at a constant low temperature, a constant velocity only after half an hour or even after several hours (Bělehrádek (1928 a, c, 1929)). This time-factor in the action of low temperatures, explanation of which will be given further on, was fully neglected before, and many results are necessarily affected by this important source of error.

It is further necessary to point out that in some instances the expression x in the equations (14, 15) ought to be corrected in the sense that the temperature should be counted from the biological zero. In most plants and cold-blooded animals, there is but a slight difference between the biological zero and 0° C., and x may be therefore taken here as identical with the ordinary temperature. But when the biological zero lies above 0° C., the observed values of times at lower temperatures are higher than the calculated ones (y), and when the biological zero is underneath 0° C., the observed times at lower temperatures lie below the theoretical curve.

If the ordinary temperature is designed as t and the difference between the biological zero and the zero Centigrade as ∞ , one has:

$$x - t = \infty \quad \dots\dots (18),$$

¹ These authors think that the formula is identical with the empirical equation of Esson for chemical reactions, with the substitution of the Centigrade temperature for the absolute one. They state that "it is difficult to conceive a cogent reason, theoretically, for the proposed change." There is, however, a serious reason for such a change, namely, that biological reactions invariably are stopped at or in the vicinity of 0° C. and not at -273° C., as already mentioned above.

and, by substitution in the equation (14):

$$y = \frac{a}{(t - \infty)^b} \quad \dots\dots(19).$$

In the previous publication of the writer (Bělehrádek (1929)), Buccianti's (1927) experimental data on the velocity of prophase in the mitosis of chick myoblasts are analysed by means of this corrected formula (19). Observed values adhere well to the theoretical curve, when the temperature is counted from $+12^\circ$ onwards ($x = t - 12$). Fig. 6 of the present review is another example taken from the experiments of Mattheai (1905) on the assimilation of the leaves of *Prunus laurocerasus*. On the assumption that $\infty = -10^\circ$ (there was still a measurable assimila-

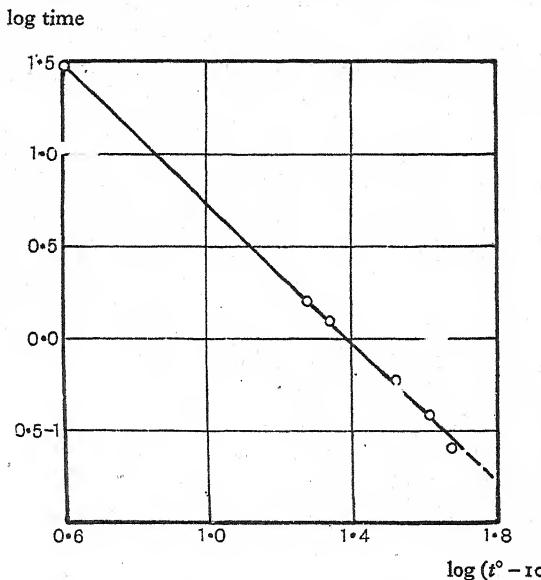


Fig. 6. Experimental data of Mattheai (1905) on the velocity of plant assimilation agree well with the proposed formula, when the zero is assumed to lie at -10° C.

tion at -6°), the experimental data may be expressed with a sufficient accuracy by the formula (14).

The mathematical value of b lies between 0.6 and 4.0 in most cases, and very often it lies very closely to 1.0. When $b = 1$, the equation becomes:

$$y = a/x \quad \dots\dots(20),$$

or

$$x \cdot y = a \quad \dots\dots(21),$$

which is identical with the equations (8, 11), expressing the rule of thermal summation and the Krogh's linear temperature formula. Thus the rule of thermal summation and the Krogh's relation are only special cases of a more general interrelation between the temperature and the velocity of biological processes, which is expressed by the formula proposed by the writer (14). This special case is realised, when $b = 1$.

Altogether it may be said that this formula has the disadvantage of being empirical, but that it is nevertheless able to do service to biologists.

In spite of the rather numerous temperature formulae in biology, there are none which can be said to hold good in every case, neither is there any rational temperature formula for biological processes. In the following pages different temperature formulae will be made use of, but it is necessary to make it clear beforehand that none of them is to be taken for more than a first empirical approximation. With this restriction, we shall use any formula which furnishes a temperature coefficient remaining constant, in the given instance, at all the temperatures concerned. This applies also to the Van't Hoff-Arrhenius equation, which, in some instances and for narrow zones of temperature, may prove useful as a descriptive and empirical formula, as long as its temperature characteristic remains constant with the temperature. The same applies also to the Q_{10} rule to a still more restricted extent.

III. MEANING OF TEMPERATURE COEFFICIENTS IN BIOLOGY.

(a) *Attempts at a classification of biological processes.*

We have seen that different formulae have been proposed to express the action of temperature on biological processes and that the majority of them have failed to bring any real progress into the problem. This failure was due (1) to the inadequacy of the formulae used in biology, and (2) to the one-sided views which as yet dominated the question.

Some authors, especially Snyder (1907, 1908), J. Loeb (1907, 1908) and Bernstein (1908), hoped that it would be possible to classify and to identify biological processes and the underlying biochemical or physical reactions on the basis of their temperature coefficients. These hopes have been fulfilled only in one case, namely that of photochemical reactions, which are practically independent of temperature and thus have a low temperature coefficient ($Q_{10} = 1.0$, $\mu = 0$, $b = 0$). Many biological reactions and many actions of radiations upon the living matter show such a temperature coefficient and they were thus revealed as being of photochemical nature (Henri et Henri (1912), Hecht (1919, 1920, 1926), Dognon (1926)).

There is, however, no absolute certainty whether the underlying process really is photochemical or not, because any thermoneutral reaction, *i.e.* a reaction in which no heat change is involved, is similarly independent of temperature.

The killing of protoplasm by heat, on the contrary, shows very high temperature coefficients (for the bibliography, see Kanitz (1915) and Przibram (1923)). This was compared by some authors (Arrhenius (1908), Kanitz (1907 a, 1915) and others after them) to the destruction (coagulation) of enzymes and colloids by high temperatures, where similar high temperature coefficients occur. J. Loeb (1908) stated that the killing of organisms by high temperatures is accompanied by a high Q_{10} , while the normal development gives a much lower value ($Q_{10} = 2-3$). Hence this author concluded that the process of senescence and of death has a different biochemical

basis from the process of development. This conclusion, however, is not justified for several reasons. Child (1915) emphasised that death by heat and normal death following senescence are two widely different processes and that the Loeb's conclusions cannot concern normal death. Lepeschkin, who first seemed inclined to accept Loeb's views (Lepeschkin (1912)), recently found (Lepeschkin (1923, 1924)) that the injury caused by high temperatures may be repaired when the cell is left for some time in normal conditions. It again follows that the thermal death is different from the natural one, because at high temperatures no similar reparation can take place, the destruction proceeding too quickly. Furthermore, the death of isolated nerves, muscles and other organs at ordinary temperatures is accompanied by a much lower temperature coefficient than the death of protoplasm by heat (see Galeotti and Porcelli (1911), Amberson (1922), Scaffidi (1926)). The writer has found in collaboration with Melichar (unpublished) that the times of survival of isolated leaves of the *Elodea canadensis* at various temperatures give a high temperature coefficient at high temperatures and a low one at lower temperatures. Therefore the assumption made by Loeb (1908), shared also by Moore (1910) and originally too by Lepeschkin (1912), that the duration of life under normal temperature conditions could be obtained by a simple extrapolation of the heat death curve, cannot be maintained.

In some instances, when the underlying process is based upon surface energy, the temperature coefficient is negative. This case is realised, according to Bernstein (1908), in the energy of muscular contraction. The temperature coefficient of any biological reaction of course becomes negative at temperatures lying above the optimum, even when no surface phenomena can be assumed to take place or to play the rôle of master processes.

Many investigators found that the Q_{10} of numerous biological phenomena is lower than 2 and higher than 1 and that it is chiefly at higher temperatures where this is observed, while the same process may give a Q_{10} higher than 2 at lower temperatures. Originally this phenomenon was explained by the assumption that some biological processes depend on the velocity of diffusion, which thus plays the rôle of limiting factor. As a matter of fact, Nernst (1904) and Brunner (1904) showed that the reaction velocity of chemical processes, taking place in a heterogeneous system (a) depends on the rate of diffusion only, and (b) gives a low temperature coefficient, as the rate of diffusion is but little affected by temperature (Brunner (1904)). From this a discussion arose among the biologists, whether or not it would be possible to conclude from the mathematical value of Q_{10} that a given biological process is based on a "chemical" or "physical" reaction (see especially Snyder (1907), Sutherland (1908), K. Lucas (1908), Pütter (1914), Warburg (1914), Kanitz (1915), Osterhout (1917)). Some authors assumed that what is called "biological process" is a catenary series of many processes, each of which has its own temperature coefficient (see above) and that it would be possible to identify these master processes by their temperature coefficients. But it may be said to-day that a distinction between "chemical" and "physical" processes as well as a classification of master reactions is not possible, because (a) there are even numerous

chemical phenomena which give a Q_{10} lower than 2, (b) two processes of quite different nature may have the same Q_{10} (see Bayliss (1924)), (c) there is no satisfactory evidence as to whether the temperature coefficient of diffusion in highly viscous media, such as protoplasm, is low also or not.

Przibram (1923) gives a long list of biological reactions and tries to group them by their temperature coefficients. This list, however, furnishes no evidence that definite processes would be characterised by definite temperature coefficients—except those mentioned above. In addition to the reasons which have just been cited, the chief cause thereof is undoubtedly the inconstancy of Q_{10} at various temperatures. Even if it were possible to undertake a classification on the basis of temperature coefficients, the Q_{10} is too rough and too inaccurate a quantity to serve such a purpose.

It is undoubtedly for this latter reason that Crozier with his collaborators (Crozier (1924 a, b, c), 1926 a, b, c), Crozier and Federighi (1924 a, b), Crozier and Stier (1925, 1926 a, b, 1927 a, b)) attempted once more to undertake a classification and identification of biological master-reactions on the basis of a quantity which seemed to these authors to be more accurate in biology, namely by means of the temperature characteristics.

Crozier finds that the majority of the biological phenomena depend on the temperature in such a way that for different temperature zones definite values of μ may be found, which appear suddenly at "critical temperatures." The temperature-velocity curves therefore are not continuous, but show sharp breaks at these temperatures. Crozier further finds that the same values of μ occur in the most diverse biological processes and concludes that at a definite temperature the velocity of a given biological process is limited by the velocity of a master reaction, which thus may be detected by means of its μ .

We have seen above that the value of μ really depends on the temperature and have explained the reason thereof. But its change with the temperature takes place gradually and there are in reality no breaks of the temperature-velocity curves, as Crozier maintains. From some graphs, as for instance from that of Brown (1926) regarding the duration of an instar in cladocerans, it is evident that one smooth curve ought rather to be drawn through the observed points instead of three separate curves, crossing at "critical temperatures," which appear thus as artefacts. Crozier (1926 a) indicates that the following are the most frequent critical temperatures: 4°, 5°, 9°, 15°, 20°, 27° and 30°. Their number alone makes Crozier's statements doubtful. Furthermore we have seen that even in simple enzymatic processes, where there can be no question of master reactions, the μ steadily decreases in value with increasing temperature.

In addition to it, the value of μ in one and the same biological process may vary with the age of the organism, as is evident from Murray's (1926) and Cohn's (1928) data for the heart-beat frequency in the chick embryo. Similar systematic variations of μ with age are not consistent with the idea that the temperature characteristics of biological reactions are indicators of their biochemical bases, but may easily be explained in another way; as will be shown further on.

Crozier's idea is based on a mistake and his attempt at a classification of biological reactions cannot therefore have any success.

(b) *Temperature coefficients as indicators of protoplasmic viscosity.*

There can be no doubt that the temperature question in biology has quite a different aspect from the analogous problem in chemistry, and that any application of chemical laws on biological phenomena is here necessarily premature and misleading. This is chiefly due to the fact that living matter is a system of manifold phases, separated by surfaces and characterised by different chemical and physical properties, in which viscosity (consistency) seems to be of the first importance. By various viscosimetric measurements, the technique of which has been reviewed by Weber (1924), many authors have shown that protoplasm is a viscous stuff, whose viscosity may vary to a considerable extent, and more recent investigations, made by means of the microdissection method, have demonstrated that various protoplasmic phases (cellular constituents) differ greatly as to their consistency. This important feature of protoplasm has not yet been sufficiently explored from the physico-chemical point of view. Yet there is a close relation between viscosity and rate of diffusion, as shown by the Einstein-Smoluchowski formula:

$$D = \frac{kRT}{N} \cdot \frac{1}{6\pi\eta r} \quad \dots\dots(22),$$

where D is the diffusion coefficient, R the gas constant, N the Avogadro's number, r the radius of the diffusing particle, η the viscosity and k a constant.

Nernst (1904) and Brunner (1904) showed that the velocity of a chemical reaction in a heterogeneous system depends on the rate of diffusion only, as the diffusion is the slowest process amongst those which together form a "reaction in heterogeneous system," and is therefore the master process of the whole. This principle, which has been applied to biological reactions chiefly by W. M. Bayliss (1918), states that the velocity of biological processes is governed by the rate of diffusion. Bayliss, however, refuses to conclude that this is always the case and cites an example, namely the reaction between an acid and solid metal, where the diffusion is quicker than the chemical process alone. Reactions of this type of course will be of the rarest occurrence in living protoplasm. On the contrary, the rate of diffusion in protoplasm is probably very low, as the viscosity often is high and the radius of the reacting molecules great (see equation (22)). Sutherland (1908), Henri and Henri (1912), Warburg (1914), Osterhout (1917), Dhar (1920), Przibram (1923) have also emphasised the fundamentally important part which is probably played in many cases of temperature action in biology by diffusion or by viscosity. More recently Heilbrunn (1925) and Buccianti (1927) laid stress on the heterogeneous nature of living matter and its probable importance in the action of temperature. Pantin (1924) and Fauré-Fremiet explain some special features of biological temperature action by the assumption that changes in the protoplasmic viscosity are largely involved in it.

But one will certainly be not very far from the truth when assuming that the velocity of biological processes is always or almost always governed by the rate of

diffusion in the reacting protoplasmic phases and that it is thus determined by the viscosity of the one of these phases (Bělehrádek (1926 c, 1929). This is the more probable, as Achalme and Bresson (1911) have demonstrated that the velocity of enzymatic reactions *in vitro* is closely dependent on the viscosity of the medium and as Callow (1915) has shown that the velocity of hydrolysis of methylacetate by means of HCl is reduced when it takes place in gelatin. These important statements, recently confirmed by Colin and Chaudun (1929), seem to have fully escaped to Crozier's attention. Yet they are of the first importance in biology, because on their basis the arrest of some biological processes at temperatures above 0° C. may be explained and because it strongly supports the hypothesis that the rate of protoplasmic reactions depends on the viscosity. It is probable that temperatures near zero Centigrade and sometimes much above it arrest the activity of protoplasm because the consistency of the reacting phases reaches more and more that of a solid body, so that any diffusion is made impossible or at least slow enough to hinder the reaction (Bělehrádek (1928 a, c, 1929)). These facts therefore strongly support the hypothesis that the temperature coefficient of a reaction which is based on an enzyme action, or, more generally, which takes place in a heterogeneous system, is identical with the temperature coefficient of the rate of diffusion and thus with the temperature coefficient of the viscosity of the reacting phases.

There is, however, no direct evidence that this really is so in living matter. Yet the hypothesis is greatly supported by the work of Nernst, Brunner, Achalme and Bresson, Callow, Colin and Chaudun (cited above) as well as by the work of certain biologists, showing a progressive decrease of protoplasmic viscosity with increasing temperature (see the review by Weber (1924) and by Fauré-Fremiet (1925)). The writer has brought other indirect evidence in support of this idea in the phenomenon of progressive slowing of biological reactions by a constant low temperature. He has shown (Bělehrádek, 1928 a) that the heart beat of *Daphnia*, kept at a constant temperature of + 4.5° C., acquires a constant frequency only after one hour or more, although the temperature equilibrium, measured thermo-electrically, is reached in the first few minutes of the experiment. In old grown individuals this progressive slowing may lead to a complete stop, which is perfectly and quickly reversible even when it lasts about one hour. A similar time factor has been established for the heart frequency of young trout larvae (Bělehrádek (1928 c)). It is interesting to note that the original frequency in both cases reappears without any time factor, when the animals are heated again to the original temperature. This phenomenon points out to the explanation that the velocity of heart beat is governed by some processes, which at a new (low) temperature reaches a new equilibrium with a considerable time factor. This process probably is the diffusion in a viscous medium. It is a well established fact that the viscosity of many hydrosols is changed by temperature with a similar time factor (see J. Loeb (1922)), and from the experimental data, furnished by Weber and Hohenegger (1923), the same seems to be true for the viscosity of protoplasm.

This undoubtedly is in the closest relation to a phenomenon, recently designed by Artom (1928) as "thermal hysteresis." It is known that the velocity of a given

biological process at one and the same temperature may depend on whether this temperature has been reached by heating or by cooling. The cause of this probably lies in the considerable time factor which accompanies the attainment of a velocity equilibrium at low temperatures and which is practically absent when the system is heated up again.

Temperature coefficients of biological reactions may therefore be taken for temperature coefficients of the viscosity of the reacting phases. This conclusion, of course, demands a slight correction, because the rate of diffusion on the one hand and the viscosity on the other hand are not absolutely identical functions of the temperature, as follows from the Einstein-Smoluchowski formula (22).

An analysis of J. Loeb's (1922) data on the action of temperature on the viscosity of isoelectric gelatin at different concentrations shows (Bělehrádek (1929)) that (a) the values obtained agree with the equation proposed by the writer (14, 15) and that (b) the temperature coefficient b increases with the concentration of gelatin solution. The following table clearly demonstrates this:

Concentration of gelatin, per cent.	b	Concentration of gelatin, per cent.	b
0·25	0·04	2·0	0·36
0·5	0·07	2·5	0·46
1·0	0·20	3·5	0·53
1·5	0·29		

It follows that the temperature coefficient of the viscosity is a function of the concentration, or of the viscosity itself, considered at a constant arbitrary temperature.

This conclusion, when applied to what has been said previously regarding the living substance, leads to the hypothesis that the temperature coefficients of identical biological processes are indicators of the viscosity of reacting phases.

This hypothesis, already published by the writer in previous papers (Bělehrádek (1926 c, 1928 a, 1929)) can be tested on some biological data furnished by different authors. If it is true that the temperature coefficients are expressions of the viscosity of phases involved in a given reaction, it is to be expected that their numerical value will undergo changes whenever the protoplasmic viscosity varies.

(c) Variations of temperature coefficients with age.

Important variations of protoplasmic viscosity, measured directly by different methods, have been shown to occur as a function of age. Generally speaking, there is a steady increase of the viscosity with age in animal and plant protoplasm (see Weber (1924), Bělehrádek (1925)). So far as one and the same biochemical process is concerned, one should therefore find corresponding variations of temperature coefficients, if the hypothesis put forward by the writer holds good.

As a matter of fact it is not difficult to find in the earlier as well as in the more recent literature experimental data which agree fairly well with this hypothesis and which have not yet been fully explored in an appropriate way.

The first who seems to have noticed that the Q_{10} varies with age was Cesana (1912). This author experimented on the action of temperature upon the heart beat

frequency in chick embryos and found that the numerical value of the Q_{10} is not equal for embryos of different ages, but that it markedly increases with their age. The following table gives Cesana's figures:

Age of embryo	Q_{10}	Age of embryo	Q_{10}
44 hours	1.18	4 days	1.30-1.47
49 "	1.36	5 "	1.76
58 "	1.27	6 "	1.83-2.63
70 "	1.30	7 "	2.08-2.91
72 "	1.44	8 "	2.04-2.57
73 "	1.30-1.40	9 "	2.00
74 "	1.40-1.42	—	—
76 "	1.14	—	—
79 "	1.50	—	—

As Cesana used the Q_{10} rule, his results do not show an absolute regularity. In spite of this the progressive increase of Q_{10} with age is evident, because the range of temperatures this author worked at is narrow. Cesana explained this result vaguely by physiological changes which take place during development, but he did not closer define their nature.

Temperature action on the embryonic heart of the chick has recently been studied by Murray, jr. (1926) and by Cohn (1928). While Murray, jr. (1926) experimented with isolated heart fragments, Cohn's experiments were performed on whole hearts *in situ*. Murray, jr., showed that the value of μ , which for temperatures between 32°-40° C. may be used as an empirical constant, varies in this case to a considerable extent with age, but it is evident from his figures that the auricle and the ventricle behave just in opposite manners:

Age of embryo (days)	μ of auricle	μ of ventricle
3	25,500	—
4	21,300	10,900
5	13,400	17,100
6	8,000	—
8	—	19,500

Similarly Cohn's experiments show a regular decrease of temperature coefficients with the age of embryos. There is, however, some discordancy between the results of these two authors on the one hand and those of Cesana on the other as to the direction of change of temperature coefficient with age.

Similar variations of temperature coefficient with age are evident in the heartbeat of larval *Ambystoma*, as studied by Laurens (1914). This author, too, used the Q_{10} rule and his figures, given in the following table, are average values of Q_{10} , calculated for successive temperature zones between + 5° and 30° C. In the third column the temperature constant b , calculated by the writer, is added.

Length of the larva (mm.)	Q_{10}	b
8.0-8.4	2.39	1.40
8.9-9.1	2.39	1.36
12.5-13.5	2.43	1.50
24.0-27.0	2.51	1.50

Here, too, the temperature coefficients show a rise with the age of embryos. Laurens himself did not lay stress on these variations which he probably attributed to experimental errors.

Another example of the same kind is furnished in a recent paper by Andersen (1929) on the action of temperature upon the embryonic heart rhythm in the lizard (*Lacerta agilis* L.). Andersen states that the Q_{10} is constant only up to 18° and that it depends on the age of embryos. The following are Andersen's figures, together with, in the fourth column, the values of b (which are fairly constant between 10° and 38°).

Group	Length of embryos (mm.)	Q_{10} ($15-18^\circ$)	b ($10-38^\circ$)
I	5	4.6	1.88
II	9.5-10	5.7	2.02
III	11	6.3	2.40
IV	11.5	6.2	2.40
V	18-19	6.1	2.40

Here the variations are such that they could not be ascribed to experimental errors.

Crozier and Stier (1927 b) state that the frequency of cardiac contractions in *Limulus* gives different values of μ in embryonic and adult hearts and explain this phenomenon by the assumption that the essential controlling biochemical processes are unlike in the embryo and in the adult animal. These authors are inclined to explain in a similar way also every variation of μ with age. They declare (Crozier and Stier (1927 a)) that they have never observed any systematic variations of μ , which would be in accordance with the writer's hypothesis. Yet it is easy to calculate temperature coefficients from the experimental data of Crozier and Hubbs (1924) on the cardiac rhythm of *Leucichthys artedi*, which seem to show a systematic increase with age (see Bělehrádek (1929)):

Stage	b
A	0.76
D	0.94
G	1.08

In addition to experiments dealing with the action of temperature on the cardiac rhythm of different animals, certain observations regarding ontogenetic processes may also be cited in support of the views developed here.

Regener (1865) studied the influence of temperature on the development of *Dendrolimus pini* L. and found data from which the following values of b are calculated for different stages: embryo, $b = 0.50$; larva, $b = 1.80$; pupa, $b = 2.00$. Similarly, important variations of the temperature coefficient with the age may be calculated from Henning's (1907) experimental data on the development of the beetle *Tomicus typographus*: embryo, $b = 1.87$; larva, $b = 2.98$; free beetle, $b = 2.44$. In a preliminary note (Bělehrádek (1926 c)) the author has pointed out that b in some instances suddenly drops between the larval period and the imaginal stage and that this might be explained as some sort of rejuvenation (decrease of protoplasmic viscosity) during insect metamorphosis. In the light of other experimental facts, which cannot be discussed here in detail, this explanation, however, is not sufficiently founded.

Przibram (1916) indicates values of Q_{10} for successive moulting periods in *Spodromantis bioculata* Burm., which, too, show a tendency to increase with the age:

Moults	Q_{10} (25°–35°)	Moults	Q_{10} (25°–35°)
1–2	1.25	6–7	3.25
2–3	1.50	7–8	1.70
3–4	2.00	8–9	2.79
4–5	1.57	9–10	2.25
5–6	1.83	—	—

Fauré-Fremiet (1925) points out that the temperature coefficients of developmental processes are unlike for different stages and Melvin (1928) states that in the earlier stages of insect development the temperature has a lesser accelerating effect than in the later ones. According to a quotation from Ludwig (1928), Sanderson

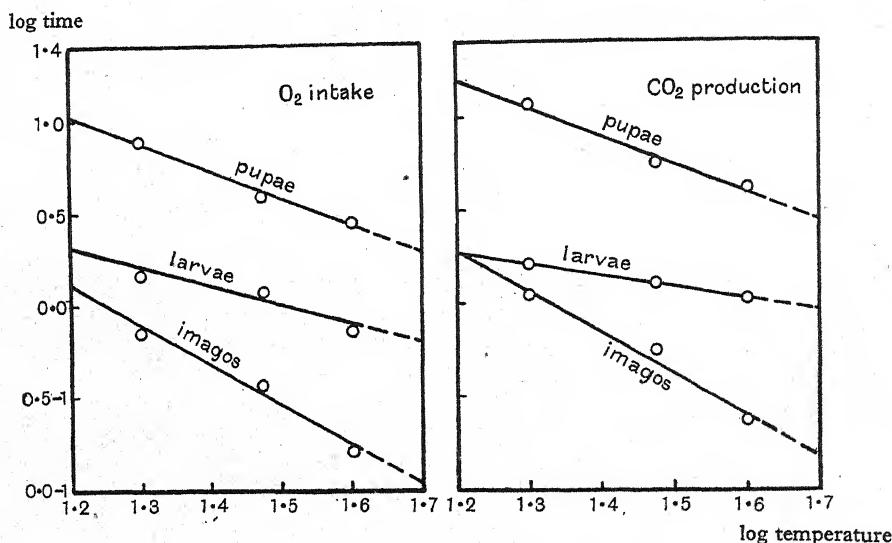


Fig. 7. Action of temperature on the oxygen intake (left) and on the CO₂ production in different developmental stages of *Bombyx mori*, according to Battelli and Stern (1913).

noticed as early as 1910 that the μ of insect development varies with age and Ludwig (1928) recently finds the same. Also Friend (1927) has stated that the temperature characteristic (μ) is not the same for the quiescent premoult period as for the feeding period in the development of the birch-leaf skeletoniser.

By far the most important experimental data with regard to the hypothesis evolved here are those furnished by Blunck (1924) on the velocity of development in the beetle *Dytiscus semisulcatus*. Not only do these data agree well with the formula (14, 15), but also the value of b regularly increases with age:

Period	b	Period	b
Embryonic...	1.10	Third larval instar...	1.38
First larval instar ...	1.14	Praepupal instar ...	1.48
Second larval instar	1.26	Pupal instar ...	1.60

From the graphs already published (Bělehrádek (1926 c, 1929)) it is evident that the increase of b with the age is progressive and gives a regular S-shaped curve, which is of common occurrence in growth phenomena. This regularity as well as the shape of the curve clearly show that the rise of the temperature coefficient with age cannot be due to alternative chemical master reactions in the sense of Crozier's hypothesis, but that its cause probably lies in a progressive increase of protoplasmic consistency with the age. Crozier (1926 c), who has analysed Blunck's figures with the aid of the Van't Hoff-Arrhenius formula, finds the same μ for all developmental stages. This, however, is explained by the inadequacy of the formula used by this author.

As to the developmental processes in vertebrates, Krogh's (1914 a) results on the early development of the frog show that here, too, b increases with age (see Bělehrádek (1926 c)).

Scarce data on the simultaneous action of temperature and age on the rate of metabolic processes clearly demonstrate a similar increase of b with age. From the figures of Battelli and Stern (1913), whose graphical representation by means of the formula (14, 15) is given in Fig. 7, the following values of b are calculated:

	b (oxygen intake)	b (CO ₂ production)
(a) <i>Bombyx mori</i> :		
Larva	0.72	0.60
Pupa	1.44	1.52
Imago	2.12	2.24
(b) <i>Musca domestica</i> :		
Larva	1.50	1.70
Pupa	1.56	1.84
Imago	1.84	1.94

As to plant metabolism, Bělehrádek and Bělehrádková (1929) found that the rate of oxygen intake in leaves of *Scolopendrium scolopendrium* Karst. gives in the youngest leaves $b = 1.11$, in the medium sized leaves $b = 2.18$ and in the oldest ones $b = 0.88$.

(d) Variations of temperature coefficients with other factors.

By direct measurements on the plasmodia of *Myxomycetes*, Heilbronn (1922) demonstrated that loss of water by drying causes an increase of protoplasmic viscosity. It is thus to be expected that in insects, which are known to undergo important variations in their water content with the relative atmospheric humidity, b will alternate with the latter. This assumption is confirmed by the experiments of Hennings (1907) on the development of *Tomicus typographus* in dry and in humid atmospheres. From his results the following figures are calculated:

	b in dry atmosphere	b in humid atmosphere
Embryonic stage...	2.02	1.87
Larval stage ...	3.52	2.98
Free beetle ...	2.90	2.44
Total development	3.48	2.37

A number of authors, whose work has been summarised by Fauré-Fremiet (1925) and by Heilbrunn (1928), have shown by various methods that the protoplasmic viscosity undergoes periodical changes during the mitotic process and that it is high at the beginning and low to the end of the process. In accordance with these direct measurements, the temperature coefficient of duration of the successive periods in mitosis was found to vary. This is apparent from Jolly's experimental data and has been recently found also by Ephrussi (1926). The starting of mitosis is accompanied by a high temperature coefficient, while the stage of monaster gives a relatively low value. Fauré-Fremiet (1925), who quotes Jolly's experiments, explains these variations of Q_{10} by the possible changes of protoplasmic viscosity during the mitotic process and Buccianti (1927) is inclined to accept the same explanation. The results of direct viscosimetry, referred to above, are thus in a perfect agreement with these variations of temperature coefficient, thus giving another support to the hypothesis put forward by the writer.

IV. SUMMARY.

(a) The problem of temperature coefficients in biology was initiated by chemists and has suffered from the beginning from this circumstance. Attempts to apply chemical temperature-velocity formulae (the Q_{10} rule and the Van't Hoff-Arrhenius law) to biological processes failed, because none of the temperature constants used in chemistry (Q_{10} , μ) can be said to hold good in biological reactions. It is shown that the value of μ in the Van't Hoff-Arrhenius law varies, in biological processes and in simple enzyme reactions, with the temperature to about the same extent and for the same reasons as the value of Q_{10} , these constants being closely related for the zone of biokinetic temperatures. The use of the Van't Hoff-Arrhenius formula in biology, sponsored of late mainly by Crozier, is therefore of no greater advantage than the use of the simpler Q_{10} formula, both of them presenting the same low degree of accuracy in biology. A new empirical temperature formula, proposed by the writer, is shown to agree in many instances with sufficient accuracy with observed data. The constant of this formula (b) remains independent of temperature even in those cases in which the constants Q_{10} and μ show a steady decrease in value as the temperature increases. When $b = 1$, the formula becomes identical with the linear relation of Krogh and with the rule of thermal summation, which are thus special instances of a more general interrelation between temperature and the velocity of biological phenomena.

None of the temperature formulae proposed up to the present in biology can be said to hold good in every case, nor is there a rational temperature law in biology. Even the constants Q_{10} and μ are nothing more than empirical and descriptive quantities, which might be used with this restriction so far as they remain relatively constant for the temperatures concerned.

(b) The reason for which the velocity of biological phenomena is influenced by temperature in a way widely different from that commonly found in chemical reactions seems to lie in the heterogeneous nature of living matter and in the relatively high viscosity of the reacting protoplasmic phases. Even in enzyme

reactions the formulae cited above proved unsatisfactory and one cannot expect that formulae which are not accurate for enzyme reactions should yield better results in biological processes.

Some earlier attempts at a classification of biological phenomena and the underlying "master processes" by the aid of the temperature coefficient Q_{10} , as well as a more recent attempt by means of the temperature characteristics μ (Crozier), has necessarily failed for a double reason. First, the formulae used up to now in biology are so inaccurate that they do not permit of any serious analysis. "Breaks" in biological temperature curves at definite "critical" temperatures (Crozier) are artefacts, due to improper use of the chemical temperature formulae in biology, and they do not exist in reality. Second, it is highly probable that diffusion is always or almost always the master process in biological reactions and that thus the viscosity of reacting protoplasmic phases is always or almost always the limiting factor which determines the velocity of biological processes. This thesis is supported by some experimental data from the physico-chemical literature. If this is so, then the temperature coefficients of biological phenomena are merely temperature coefficients of the viscosity of reacting protoplasmic phases.

(c) If this hypothesis is correct, it follows that temperature coefficients of analogous biological processes are indicators of protoplasmic viscosity. This is made probable by an analysis of J. Loeb's data on the viscosity of gelatin solutions, which shows that the temperature coefficient of viscosity regularly increases with concentration.

The hypothesis can be tested on abundant numerical material, furnished by numerous investigators. It is shown that the temperature coefficients increase when a rise in protoplasmic viscosity may be assumed to take place. This is illustrated by the following facts: (1) the temperature coefficients of the heart-beat frequency in various animals, of the developmental processes in vertebrates and invertebrates, and of the metabolic rate of animals and plants vary systematically with the age of the organisms; (2) insect development in dry air gives a higher temperature coefficient than when it takes place in a humid atmosphere; (3) the temperature coefficient of successive periods of the mitotic process changes in accordance with the variations of protoplasmic viscosity which have been shown to take place during mitosis.

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CHROMATOPHORES

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(Received August 19, 1929.)

(With Five Text-figures.)

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I. INTRODUCTION.

THE remarkable colour changes that many animals show did not escape the attention of the naturalists of antiquity. Aristotle, often called the Father of Natural History, stated in the second book of his *Historia Animalium* that the chameleon may change from black to green or the reverse, and that in death it assumes a greenish tint. He likewise remarked in the seventh book of the same work that the octopus in seeking its prey so changes its colour as to resemble that of its surroundings, a phenomenon which it also exhibits when it is alarmed. Thus some of the most striking facts of the colour changes in animals were recognised as early as the fourth century B.C. by this acute Greek natural historian.

Many other observations, some correct and some erroneous, were added by the ancients to this field of investigation. Theophrastus, the successor of Aristotle, is quoted by Plutarch as holding the opinion that the chameleon changes its colour in consequence of emotional states, and Antigonus of Carystus seems to have originated the mistaken idea that this animal always reproduces the colour of its immediate surroundings in the tints of its own skin. The ancients recognised colour changes not only in the chameleons and in the devil-fishes but also in ordinary fishes. Thus Pliny in his *Natural History*, book ix, chapter 30, was led to remark that "masters in gastronomy inform us, that the mullet while dying assumes a variety of colours and a succession of shades, and that the hue of the red scales growing paler and paler gradually changes, more especially if it is looked at enclosed in glass." Seneca, in book iii, chapter 100, of his *Physical Investigations*, observed to the same effect that

a "mullet even if just caught is thought little of unless it is allowed to die in the hands of your guest. They are carried about enclosed in globes of glass and their colour is watched as they die which is changed by the struggles of death into various shades and hues." Chameleons, octopuses and fishes such as the mullets were the three sets of animals known to the ancient world for their colour changes, nor was this list substantially increased till about two centuries ago.

In 1715 Vallisnieri recorded for the first time the fact that the common European frog, *Rana esculenta*, could change its tint, and in 1758 Roesel von Rosenhof made a similar observation on the tree-toad. Thus amphibians were added to the list of those creatures that exhibit colour changes. Vallisnieri likewise pointed out that among reptiles in addition to the chameleon one of the European geckos also possessed the capacity of changing colour. In 1830 Stark noted that several European fishes, namely, *Leuciscus phoxinus*, *Gasterosteus aculeatus*, *Cobitus barbatula*, and *Perca fluviatilis*, became dark on a dark background and light on a light one. In 1842 Kröyer discovered the colour changes in the crustacean *Hippolyte* and thus added arthropods to the list of chromatically active animals. This discovery completed the initial steps indicative of the five important groups of animals in which colour changes occur: among the invertebrates, the cephalopods and the crustaceans; and among the vertebrates, the fishes, the amphibians, and the reptiles.

The means by which animals change their colours first excited the serious attention of investigators in the early part of the last century. Cuvier in 1817 postulated an inward and outward flow of coloured fluid in the skin of the cephalopod as a means of colour change. This hypothesis was overthrown by Sangiovanni's discovery in 1819 of the true chromatophores in these animals. He introduced the word chromatophore (*cromoforo*), described what he called the diastole and systole of these organs, and believed them to be under the influence of the nervous system. In 1829 delle Chiaje saw for the first time the radial fibres by which the chromatophores in cephalopods are moved. In 1831 van der Hoeven made some important but incomplete observations on the mechanism of colour change in the chameleon in which he attributed much to the movement of the black pigment. But the first clear understanding of this process dates from 1834 when Milne-Edwards showed that the skin of the chameleon contained two classes of coloured material: peripheral masses of light-tinted, immobile pigment which is either exposed to view or covered up by motile black pigment. The black pigment, according to Milne-Edwards, was contained in deep-seated saccules provided with many peripheral branched extensions. These saccules or utricles, as they were called, are the chromatophores of modern workers. Milne-Edwards may therefore be said to have discovered these bodies in reptiles and to have indicated correctly their general method of action. The chromatophores of amphibians were first discovered and described in the frog by Ascherson in 1840; those of fishes by von Siebold and by Buchholz independently in 1863; and those of the crustaceans by Sars in 1867.

Before the middle of the last century the work on the colour changes in animals was of a somewhat desultory and pioneer character, but in 1852 Brücke published a monograph on these activities in the African chameleon; this publication set an

entirely new standard for such investigations. In consequence, the second half of the past century witnessed a great advance in the quantity and quality of the work in this field with the result that much new material was added and many novel problems arose. Shortly after the opening of the present century the whole field of colour changes was exhaustively and ably reviewed in two unusual summaries; the first of these was published in 1906 by van Rynberk and the second in 1914 by Fuchs. Both make present reference to the earlier literature superfluous. Consequently, with few exceptions, the articles discussed in the following pages will be those published since the date of Fuchs' summary, and the reader is referred to the reviews just mentioned for earlier papers. An excellent though brief survey of the whole subject is to be found in Hogben's recent book entitled *The Pigmentary Effector System*.

Chromatophores were believed by the earlier workers to be controlled in their movements either through nervous action or through the direct influence of the environment. In 1898 Corona and Moroni showed that adrenalin, when introduced into the circulation of the frog, had a profound effect upon the chromatophores of that animal. This unique observation was subsequently confirmed by Lieben (1906), who made an extended investigation of the subject. Comments by Fuchs (1914) on these two pieces of work led Redfield (1916) to investigate the effects of this hormone on the chromatophores of the lizard, *Phrynosoma*, with the result that adrenalin was found to be a potent agent in inducing chromatophoral changes. Since Redfield's work, much has been done on this aspect of the subject, and thus a new chapter on the physiology of colour change has been initiated.

II. CHROMATOPHORES.

Chromatophores, meaning thereby pigment cells that are concerned with the colour changes in animals, are almost entirely limited to the five groups of organisms already mentioned: the cephalopods, crustaceans, fishes, amphibians and reptiles. The only other group in which true chromatophores occur is that of the pteropods (Gegenbaur, Kölliker and Müller, 1853; Gegenbaur, 1855), molluscs commonly placed among the gastropods but with certain relations to the cephalopods. The chromatic organs of the pteropods exhibit the same type of structure and action as do those of the cephalopods. The chromatophores that have been described in the pulmonate gastropods, and that have been investigated recently in *Limax* by Weber (1923) are little more than pigment cells and show only slight traces of that activity which in general is associated with parts having to do with colour change. In insects the so-called chromatophores (Schmidt, 1920 a) are entitled to this designation only on the assumption that motility is not a necessary function of such cells. The remarkable colour changes in the orthopterous insect *Dixippus* (Schleip, 1910, 1915, 1921) appear to be accomplished by means wholly unassociated with chromatophores. The change in tint shown by the sea-urchin *Arbacia* (von Uexküll, 1896) under different illuminations is apparently in no way a chromatophoral effect. Nor is there reason to suppose that chromatophores are concerned with the colour changes noted by von Lendenfeld (1883) in certain sponges. In short, excepting

the few instances among the pteropods, true chromatophores are limited to the five groups of animals already noted. In structure and function the chromatophores of certain of these groups show striking differences, and these differences clearly indicate independence of origin. Without doubt there are at least three types of organically distinct chromatophores: those of the cephalopods, including the pteropods, of the crustaceans, and of the vertebrates. These types will be considered in the order named.

III. CHROMATOPHORES OF CEPHALOPODS.

The cephalopods, including the devil-fishes, the cuttle-fishes, and the squids, exhibit what are perhaps the most remarkable colour changes of any seen in the animal kingdom. Under excitement a devil-fish may change from an ashen-grey to almost black and back again, and with effects that are described as cloud-like or shadow-like in their delicacy. The endless variety and change that such an animal may show can be compared only with sky and water. In the squids the same colour-play is observable except that it is ordinarily in tints of golden orange, red, and brown. All these truly marvellous exhibitions of colour transformation are brought about by the chromatophores.

From the standpoint of structure, the cephalopod chromatophore is the most complex of all such devices. In the resting state it consists of a central, spherical, pigmented cell, the chromatophore proper, and a surrounding system of radial muscle fibres. The central cell, which is ordinarily just visible to the unaided eye, is small in the devil-fishes and large in the squids. In *Loligo*, according to Bozler (1928), there are chromatophores of three tints, brown, red, and yellow; the brown ones are the largest and the yellow the smallest. In cephalopods generally the central cell is uninuclear, is filled with a dense mass of pigment, and is contained within a highly elastic cell-membrane. Although this membrane has been suspected of possessing a thin muscular covering, no conclusive evidence for such a structure has been brought forward. The radial fibres are attached by one end to the equator of the central cell, and radiate outward from it in the plain of the skin. In different species their number varies from six or eight to twenty or more. The blunt end of each fibre, that end which is attached to the membrane of the central cell, carries the nucleus. The farther the fibre is followed from its nucleated end the smaller it becomes. Toward its distal end it branches frequently, and these branches serve as means of attachment to the surrounding tissue. The fibres of one chromatophore overlap those from others and may, in fact, be in fairly intimate contact, one system with another. In the same chromatophore, strands of tissue have been seen to pass from one fibre to another, though, according to Bozler, these connections are of only mechanical significance. Bozler has shown that in *Loligo* each muscle fibre is provided with a peripheral layer of myofibrils which run lengthwise to the given fibre. The core of the fibre is occupied by granular sarcoplasm in which, under favourable circumstances, a system of very delicate, longitudinal fibrils can be distinguished. As was long ago shown by Hofmann (1907), each muscle fibre receives a single nerve fibre which can be traced along the length of the muscle.

The colour changes in the cephalopods are due to expansions and contractions

of the central pigmented chromatophoral cells. These movements are accomplished by the action of the radial fibres on the elastic cell membranes. When all the fibres of a chromatophore contract, the spherical central cell is drawn out into a greatly expanded disc and its coloured contents are thus made to occupy a much larger area than before. The diameter of the chromatophore, when momentarily in the form of a fully expanded disc, may be as much as twenty times its diameter as a sphere. When hundreds of such spheres in the skin of the cephalopod suddenly expand into discs, a blush of colour passes over the surface of the animal. As the muscle fibres of these discs relax, the elasticity of the cell membranes draws the central cells back again to their original spherical form, and the disc of colour is reduced to a deeply tinted dot. Thus the colour, for the moment, seems to vanish. Bozler (1928, 1929) has studied in much detail the changes in the radial muscle fibres that accompany these activities (Fig. 1). In the relaxed state the peripheral myofibrils in such fibres are distinct; the nucleus of the fibre lies in the sarcoplasm near the blunt end, and

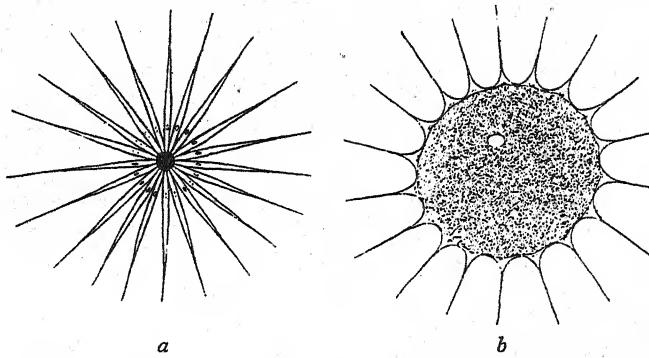


Fig. 1. Chromatophores of *Loligo*; a, contracted; b, expanded.
From Bozler, *Zeit. vergl. Physiol.* 7, 381, fig. 1, 1928.

with its long axis parallel to that of the fibre. On the contraction of the fibres the myofibrils lose their distinctness and seem to fuse into a continuous layer except at the blunt end of the fibre where they can still be distinguished. During contraction the muscle nucleus moves toward the central chromatophoral cell and changes its position so that its longitudinal axis is at right angles to that of the fibre, instead of being parallel to it. These are the more noteworthy changes that accompany the expansion and contraction of the chromatophore, and it is on the basis of these changes that the play of colours in the cephalopods is to be accounted for.

It is clear from what has been said that the cephalopod chromatophore is by no means a simple structure. The idea once advanced that it was to be considered a modified cell is quite erroneous. It is a highly organised group of cells, a central pigmented element with a radiating system of muscle fibres provided with nerves. From this standpoint the cephalopod chromatophore must be regarded as a simple type of organ, a condition scarcely reached by the chromatophores of any other animal. This exceptional state of the cephalopod chromatophore is apparent not only in its structure but also in its activities. Since each chromatophore is provided

with a group of muscles, it would not be surprising if it presented conditions indicative of such peculiarities as tonus, tetanus, inhibition, the all-or-none principle, and other functional characteristics of contractile tissue. Problems of this kind have for some time past excited the interest of investigators and have been recently worked upon by Sereni (1927 a, 1927 b), by ten Cate (1928), and by Bozler (1928, 1929).

When a normal *Loligo* in an approximately quiescent condition is watched in an aquarium, its colour changes are seen to be relatively insignificant and its chromatophores are for the most part in a state of contraction. If now it is in any way excited, the colour-play begins, and waves of characteristic tint run over its surface. If the excitement is carried to an extreme, the *Loligo* may expand to the full all its chromatophores, whereby it is rendered dark brown, and at the same instant it may discharge a cloud of ink. This appears to be the state of maximal excitation. All these activities give the impression of high co-ordination, and are usually regarded as under nervous control. If in a *Loligo*, appropriately confined on a laboratory table, a branch of nerve innervating the chromatophores of a limited area is cut, the colour-play observable on the rest of the animal ceases on the denervated area. Over this area the chromatophores take no further part in the colour-play of the rest of the animal; at first they assume a condition of full contraction, after which they pass into a semiexpanded state in which they remain almost indefinitely. If a piece of skin that will show an active colour change is removed from a *Loligo* and watched, the chromatophores in it can be seen to be at first contracted, and later partly expanded, as already mentioned. Under appropriate conditions small groups of the chromatophores on such a piece may exhibit spontaneous rhythmic movements which seem to be more or less co-ordinated. Single chromatophores will also at times show pulsations, and activity of this kind may be seen on one side only of a given chromatophoral organ. This action is sometimes so limited that it must be assumed to be produced by the contraction of a single radial fibre. On the death of a *Loligo*, the whole body of the animal whitens, and the chromatophores take on full contraction. Of the chromatophore activities in cephalopods these are some of the more obvious ones that call for elucidation.

To a number of the earlier workers in this field it seemed necessary to assume a special activity for the central chromatophoral cell in addition to its elasticity. In fact, some investigators went so far as to maintain that such cells gave evidence of a true tonus and were perhaps enveloped in a muscular sheath, but it is difficult to find ground for the justification of such ideas. It is more probable, as maintained by Bozler (1928), that the central cell of the chromatophore is nothing more than an elastic element which when left to itself assumes, through its own physical action, a spherical form. From this state it is changed only by the activity of the radial muscles which thus work against the purely elastic properties of the membrane of the central cell.

Although there appears to be no good reason for assuming tonus in the central chromatophoral cell, such a condition seems to be well established for the radial muscles. When a piece of *Loligo* skin freshly cut from the animal is allowed to stand for some time, the chromatophores after a brief period of full contraction assume,

as already stated, a condition of partial expansion, that is, the radial fibres, instead of remaining fully relaxed, contract slightly. If the preparation is left undisturbed, this condition is likely to remain till the tissue dies. If to an appropriate spot on such a preparation Faradic stimulation is now applied, the chromatophores for a considerable area about the spot respond, not with single twitches or with a tetanus, but with a more or less complete retraction showing that these radial fibres have in equal degree relaxed. The contracted condition originally assumed by the fibres is rightly believed by Bozler to be due to a peripheral tonus. Their relaxation over much of the preparation as a result of local stimulation is attributed to an excitation of inhibitory fibres in the chromatophoral nerves, for when the stimulation ceases the chromatophores of the whole field slowly expand to their original state, that is, the radial fibres again contract. Bozler concludes from these and other like observations that the chromatophoral nerves in cephalopods carry inhibitory fibres by which the peripheral tonus of the radial muscle fibres may be temporarily overcome.

If the chromatophoral nerve from a fresh piece of *Loligo* skin is stimulated by a single electric shock, the radial muscles supplied by that nerve give a single twitch as is shown by the momentary expansion of the associated chromatophores. Similar results can be obtained on direct stimulation by a single electric shock applied to the muscles of the chromatophores after their nerves have been eliminated by the application of cocaine. These reactions have all the characteristics of the single twitches of vertebrate skeletal muscle under similar treatment. If the electric stimulus, applied indirectly or directly, is in the form of a succession of shocks instead of a single one, the radial muscles assume a state of tetanus as evidenced by the continued expansion of their chromatophores. This state corresponds exactly to that seen in *Loligo* when, under natural conditions, it maintains expanded chromatophores. Bozler, therefore, believes that the chromatophoral nerves of cephalopods carry not only fibres that inhibit peripheral tonus but also those that excite tetanic contraction; in other words, he argues for a double innervation of the radial muscle fibres. In confirmation of this condition it has been shown that in their tetanic activities the radial fibres conform to the all-or-none principle, but that in their tonus no such conformity is observable.

From what has been briefly stated in the preceding paragraphs it is clear that the cephalopod chromatophore is as complicated from a functional standpoint as it is from a structural one. Its functional activities include many of the intricacies of muscle action. In its relation to the central nervous apparatus, it shows a remarkable degree of differentiation, for the whole chromatophoral system may be acted on locally in such a way as to produce the wave-like spread of colour change so characteristic of these animals. Such an activity would be impossible without a high degree of central nervous specialisation whereby a succession of superficial changes may be called forth. In this respect the cephalopod chromatophoral apparatus, peripheral and central together, must involve a complexity of communications and of controls such as are present in the modern electric sign over whose surface an ever-changing design may be made to pass. Cephalopods appear to be the only group of animals that in the evolution of a chromatophoral system have

attained to such a height. In this respect they are at most only remotely approached by the fishes.

IV. CHROMATOPHORES OF CRUSTACEANS.

The crustaceans that exhibit colour changes are found almost exclusively among the larger forms, the malacostracans, and range from the amphipods and isopods to the decapods. The first crustacean in which this activity was observed was the prawn *Hippolyte* (Kröyer, 1842), and the earliest account of the crustacean chromatophore was drawn from the schizopod *Mysis* (Sars, 1867). The systematic study of colour changes in these animals may be said to have been begun in 1872 by Pouchet, and the subject was put on a firm experimental basis by Keeble and Gamble in a succession of monographs issued during the first decade of the present century.

The crustacean chromatophore is a group of closely associated cells or perhaps better a syncitium containing a number of nuclei. The central mass is often so divided as to be suggestive of separate cells. In the expanded condition many long branching processes reach out from the central mass into the adjacent tissue. In the contracted state these processes are said not to be visible. The central mass of the chromatophore contains a dense accumulation of pigment which in expansion passes out in part into the branched processes. This pigment may be of one or more kinds. In *Crangon*, according to Koller (1927), four classes can be distinguished: dark sepia-brown, white, yellow and red. The brown is most commonly abundant and the red least so. In the movements associated with colour changes, the brown and white are more active than the yellow and red. The chromatophores are either monochromatic, in which case they are always brown; or polychromatic, brown with any one, two, or three other colours. Thus brown is found in all chromatophores, whereas the other colours occur only in combinations and less frequently. Ordinarily each branch of a chromatophore has its own colour, though combinations may also occur in the branches. In *Palaemonetes*, according to Perkins (1928), there are two classes of chromatophores, one with red and yellow pigment, and the other containing a substance that is pale yellow by reflected light and slate grey by transmitted light.

When vigorous specimens of *Palaemonetes* are put into a dish of seawater with black walls, the red-yellow chromatophores become fully expanded in about two hours, the animals assuming in consequence a dark coloration. When dark *Palaemonetes* are put into a white dish, the reverse process takes place and the animals become light. These changes are in general recognised for such crustaceans as show chromatic adjustments. The blanching of *Palaemonetes* is, however, somewhat peculiar. Two to three minutes after a dark animal has been put into a light dish its chromatophores become surrounded by a bluish cloud which sooner or later permeates the surrounding tissue. This blue coloration gradually increases for about an hour after which, with the contraction of the red and yellow pigments, it gradually vanishes. This phenomenon was observed in decapods by Pouchet as early as 1872. In certain crustaceans it is apparently a regular accompaniment of

the process of blanching. The second type of chromatophore noticed by Perkins in *Palaemonetes*, that containing the pale yellow pigment, responds to the surrounding illumination in a way the reverse of that of the red and yellow chromatophores. In a dark environment the light yellow cells are contracted and in a white one they are expanded. They are, however, so few in number in this shrimp that they have little or no effect on its general coloration. Chromatophores of this type have already been noticed in crustaceans by Pouchet (1876), Degner (1912 a), and Bauer and Degner (1913). In *Crangon*, according to Koller (1927), a more complicated form of reaction occurs. This crustacean is not only dark on a dark background and light on a light one, but it assumes an appropriate tint for a yellow, orange, or red background, thus giving indisputable evidence of a true colour sense. These several tints are produced by the movements of appropriate types of pigment.

In many crustaceans their colour changes are not only associated with the immediate environment but also with the general change from day to night; in other words, these animals exhibit a daily rhythm. This rhythm has been studied in *Hippolyte* and other forms by Keeble and Gamble. If specimens of *Hippolyte* are collected and their colours noted in the daytime, they will be found as a rule to be either brownish or greenish, depending upon the colour of the seaweed from which they have been taken. When night comes on, irrespective of the colour of the shrimp during the daytime, it assumes a transparent blue or greenish blue, and ordinarily retains this tint till daybreak. Thus day and night are characterised in *Hippolyte* by strikingly different colour phases. The rhythm of change from one colour to the other, is said not to be immediately disturbed by an experimental alteration of the environment. In continuous light or continuous darkness, the rhythm is believed to persist for several days both in *Hippolyte* and in other crustaceans (Piéron, 1913).

It is clear from the foregoing account that colour changes in crustaceans result from the migration of pigment inward and outward from a central source, the body of the chromatophore. Matzdorff (1883) believed that the cell processes containing the pigment are projected from the body of the chromatophore much as the pseudopodia are extruded from an amoeba, and that they are strictly temporary in character. If, however, a given chromatophore, as, for instance, one in *Palaemonetes*, is photographed in the expanded state and then made first to contract and later to expand again, a second photograph shows the same form of branches even in detail that the first one does. Hence the contraction and expansion of the chromatophore is not a free and uncircumscribed movement, but is a definitely limited one (Fig. 2). These results recently obtained by Perkins (1928), confirm the earlier work of Keeble and Gamble (1900), of Fröhlich (1910), and of Franz (1910), and are opposed to the opinion originally held by Matzdorff (1883). Such changes presumably result either from the migration of central pigment into the preformed processes of the chromatophore, or from the flowing of chromatophoral substance with the accompanying pigment into a system of preformed spaces. The operation, complicated enough in monochromatic chromatophores, becomes very much more so in polychromatic elements, where it is possible that as many as four sets of pigments may be in more or less independent action at once.

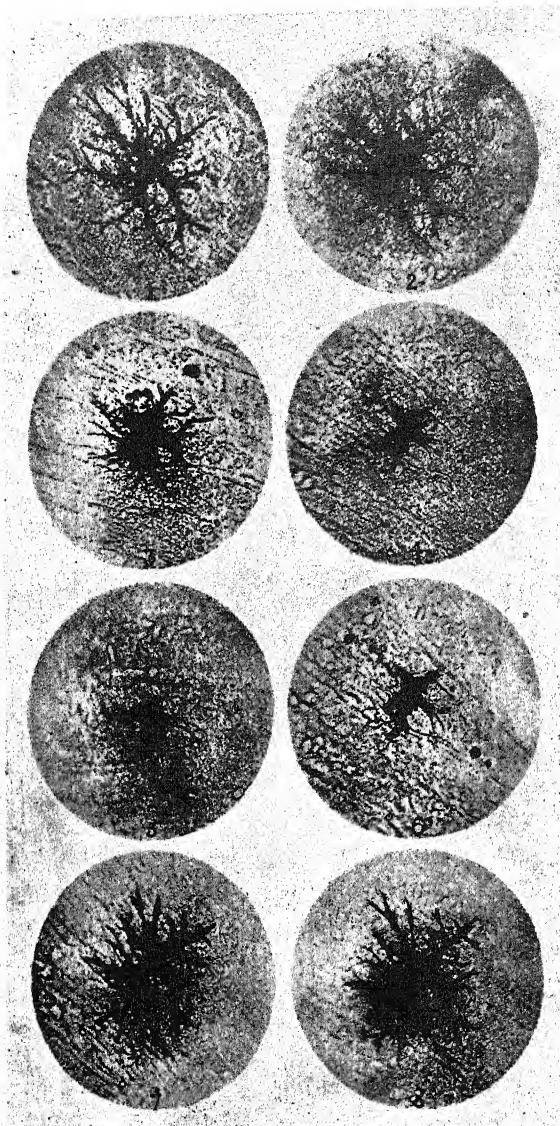


Fig. 2. Chromatophore from *Palaemonetes*; eight views of the same chromatophore at different stages of contraction and expansion. 1, fully expanded; 2, beginning to contract; 3, half-contracted after thirty minutes over a white background; 4, more nearly contracted; 5, fully contracted after two hours over a white background; 6, beginning to expand over a black background; 7, partly expanded after an hour over a black background; 8, fully expanded after two hours over a black background. From Perkins, *Journ. Exp. Zool.* 50, 101, pl. 1, 1928.

Pouchet (1872) long ago showed that the colour changes in crustaceans were dependent upon the animal's eyes. If one eye is covered or removed, the other eye is sufficient to maintain the normal colour change, but if both eyes are obstructed the changes cease. Only in *Hyperia* has Schlieper (1926), following the work of Lehmann (1923), been able recently to show that tactile organs are probably more important than eyes in initiating colour changes. In general, however, almost all later workers (Menke, 1911; Megušar, 1912; Degner, 1912 a, 1912 b) agree in assigning to the eye an exclusive rôle in this respect, a conclusion supported by the results of Koller (1925) on *Crangon*, and of Perkins (1928) on *Palaemonetes*. When one eye is removed from *Palaemonetes* there is, as might be expected, no effect on the chromatic responses, but if both eyes are cut off a complete expansion of the chromatophores follows, and this state persists irrespective of the illumination or the background. The expansion thus produced is complete in about two hours after the removal of the eyes, the normal period for such a change, and the state ordinarily lasts till the animal's death.

If a normally active *Palaemonetes* is put in complete darkness the chromatophores, if not already contracted, become so, and remain so. Thus the animal assumes in darkness a state natural to it when it is on an illuminated white background, and the reverse of that which it exhibits when it is over a lighted black background. Thus seeing black, so to speak, and being in the dark call forth opposite chromatophoral responses in *Palaemonetes*, a condition already recognised in such other animals as the fishes.

Notwithstanding the opinion held by most early workers such as Keeble and Gamble (1900), and Bauer (1905), external influences appear to have little or no direct effect on the pigment migration in crustacean chromatophores. Tait (1910) observed no effect from light directly applied to the chromatophores of *Ligia*. Koller (1927) was unable to find that heat or ultra violet radiation influenced the migration of *Crangon*, and Perkins (1928) could detect no change from the direct application of light to the chromatophores of blinded *Palaemonetes*. Practically all investigators, however, agree that the light that enters the eye of the crustacean is the means of inducing colour changes. As already shown, this conclusion is well supported by experimental evidence. In consequence of this relation, it has been generally assumed that in crustaceans, nerve tracts must pass from the eyes through the central nervous organs to the chromatophores, and there end in some form of nerve terminal. It is a remarkable circumstance that notwithstanding the fact that many investigators have sought for these nervous connections, no one has been wholly successful in finding them. Retzius (1890) described on the chromatophores of *Palaemon* what he believed to be nerve terminals. However his findings have not been confirmed by others, nor has anyone shown in an indisputable way, as has been done for the chromatophores of cephalopods and vertebrates, that nerve fibres connect with these effectors.

To demonstrate that nerves are essential to the normal action of the crustacean chromatophore, Pouchet as early as 1876 resorted to nerve cutting. His attempts were followed by those of Mayer (1879), Fröhlich (1910), and Degner (1912 a,

1912 b), but to no avail. This method was also attempted by Perkins (1928) who showed that when the ventral ganglionic chain of *Palaemonetes* was severed in the region between thorax and abdomen, no observable effect could be noticed in the subsequent colour changes. These processes continued as in a normal shrimp. Moreover, if in different shrimps various cuts are made across the abdomen near its connection with the thorax and in such ways that the cuts collectively would sever the abdomen from the thorax, none of these disturbs the colour change in the shrimp except such as pass through the dorsal blood vessel. Whenever this vessel is severed, the chromatophores, if they are not already expanded, pass quickly into that state and remain so indefinitely. If a small side branch from the dorsal vessel is cut, the chromatophores of the region supplied by that branch expand and remain permanently so, while those on the rest of the abdomen continue to show the characteristic changes. From experiments of this kind it was suspected that chromatophoral nerves may accompany the blood vessels and be distributed to these effectors along the lines of the dorsal blood vessel and its branches. When, however, these vessels were studied histologically, not the least sign of nerve fibres could be discovered on them, and Perkins was forced to the conclusion that the agent controlling chromatophore action in *Palaemonetes* was some constituent of the blood itself.

In 1925 Koller showed that if the blood from a dark *Crangon* is drawn and injected into a light one, the light shrimp quickly becomes dark. Perkins (1928) was unable to carry out on *Palaemonetes* successful experiments of this kind, but attempts were made to discover in the body of this shrimp the source of a substance that might induce such a change. It is evident from the experimental results already brought forward that the light that enters the eye of *Palaemonetes* is the outward stimulus to colour change, and that what immediately induces the change in the chromatophore is a substance carried in the blood. Where is this substance produced? Perkins tried watery extracts from many organs in the body of *Palaemonetes*, but without success. Finally he took the eye stalks from several light-coloured *Palaemonetes*, crushed them in seawater, and injected the extract thus obtained into a blinded *Palaemonetes* whose dark chromatophores in consequence of the treatment it had undergone, were expanded. Within an hour the dark pigment in these chromatophores had contracted and it remained so for about a day, after which it again expanded. No such changes were produced by the injection of seawater into the shrimp, and it was therefore concluded that when the retina of *Palaemonetes* is stimulated by the light from a white background, the eye stalks of this shrimp produce a substance that passes into the blood and excites a contraction of the dark pigment of those chromatophores to which the blood is distributed. As confirmatory of this view, Perkins succeeded by a very simple operation in closing temporarily the dorsal abdominal blood vessel. When in a light-coloured *Palaemonetes* the blood vessel was for the time being closed, the portion of the shrimp behind the region of closure became dark; on releasing the vessel again so that the current of blood was re-established, this region returned to its original light state. Although it was carefully sought for, no substance that would produce the reverse effect on the dark pigment in the chromatophores of *Palaemonetes* was found by Perkins.

These results were abundantly confirmed on several shrimps by Koller (1928) who also showed that if an extract is made from the rostral region of a shrimp, this extract when injected into an animal, the dark pigment of whose chromatophores was contracted, will cause this pigment to expand. Thus a so-called black-organ was located whose function in the production of hormones was the reverse of that of the eye stalk. Koller designated the substance produced by the eye-stalk as "contractin" in consequence of its action on the dark pigment of the chromatophores; and that produced by the "black organ" as "expantin". It is interesting to note that the "black organ" occurs in that part of the adult shrimps where the larval nauplius eye has disappeared. Perhaps some of the tissue of this primitive visual organ persists in the adult shrimp at this spot, and secretes the hormone for chromatophoral expansion. Thus both contraction and expansion of the crustacean chromatophores may be induced by hormones, contraction by the eye-stalk hormone and expansion by the rostral hormone.

If the colour changes in crustaceans are controlled by hormones, it is not surprising that all the experiments on the cutting of nerves with the view of discovering the tracts over which the effector impulses pass should have resulted negatively. Nor is it surprising that no one has found nerve terminals for crustacean chromatophores. There is apparently no good reason to suppose that chromatophoral fibres or terminals exist in crustaceans. In this respect the crustacean chromatophore is in strong contrast with that in the cephalopod. The cephalopod chromatic organ is completely under nerve control and so far as is known is uninfluenced by hormones. The crustacean chromatophore is apparently purely humoral and without nervous connections. It is evidence of this kind, in addition to anatomical differences and the like, that points to the complete independence in origin of the two systems of chromatophores thus far considered.

Although the hormone control of the crustacean chromatophores may seem to be an easy solution of this particular aspect of the general problem; it must be kept in mind that the implications of this solution are by no means simple. When the details of these relations in such an animal as *Crangon* are pictured, the complexity of the situation must be apparent. This shrimp, according to Koller, adapts itself well to a white, black, yellow, orange, or red background. Are we to assume a separate hormone or possibly a pair of hormones for each of these changes? What induces at the same moment and in the same chromatophore, the outward migration of the dark pigment and the inward migration of the white pigment? Questions like these, for which we have at present no adequate answers, show that we are as yet far from a complete understanding of operations of even such simple chromatophores as those in the crustaceans, and suggests a degree of complexity in the functions involved that strain to the limit any explanation thus far offered for the activities of these parts (Piéron, 1913, 1914).

V. CHROMATOPHORES OF VERTEBRATES.

The chameleons and mullets, as stated in the earlier part of this article, were among the animals that first attracted the attention of naturalists to colour changes. Both these forms were well known to the ancients for their chromatic activities. Frogs and toads were not added to this list till the eighteenth century when the study of their colour reactions was initiated by Vallisnieri (1715). Thus all three of the cold-blooded groups of vertebrates, the fishes, the amphibians and the reptiles, were found to have representatives exhibiting these peculiarities. It is now known that colour changes occur in many fishes, almost all amphibians and, among the reptiles, in most lizards and a few snakes. In the warm-blooded vertebrates, on the other hand, these changes seem to be entirely absent, nor is this surprising, for the skin of birds and of mammals is for the most part abundantly covered with either

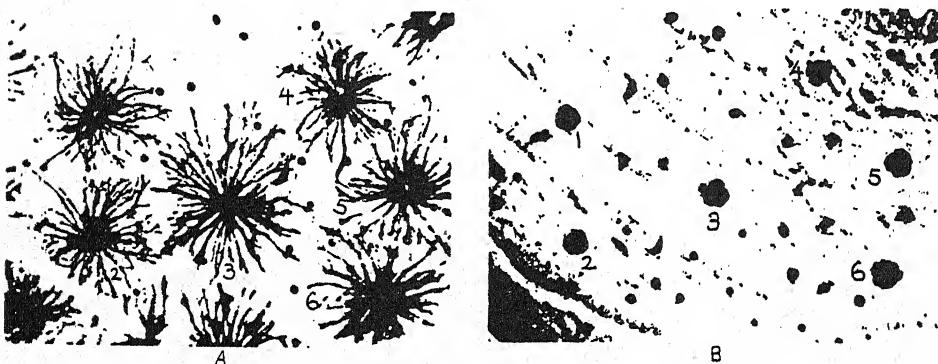


Fig. 3. Chromatophores from *Fundulus*; two views of the same group. A, fully expanded; B, fully contracted. The numbers refer to the same chromatophore in the expanded and in the contracted state. From Spaeth, *Anat. Anz.* 44, 521, Fig. 1, p. 522, Fig. 2, 1913.

feathers or hair. Colour changes, were they to occur in such animals, would be in most instances quite hidden from view.

The colour changes in vertebrates operate upon a relatively uniform plan as might be expected from the fact that the three groups in which they occur are all closely related phylogenetically. Chromatophores containing blackish pigment, the so-called melanophores, expand and thus give the animal a dark coloration, or contract and thus serve to lighten it (Fig. 3). These melanophores are commonly associated with immobile light-coloured masses of material, sometimes cellular, sometimes not, which are covered up in the expanded state of the black cells and are exposed to view in their contracted condition. Thus the light-coloured materials accentuate the light phase of the colour change. In some instances these materials are contained in cells which, like the melanophores, are motile; such cells can then expand and contract of themselves. Considerable differences exist as to the number and arrangement of these various elements in the skins of different vertebrates. In the web of the frog's foot little more than melanophores are present. On contraction

these are reduced to small black centres; on expansion they form an interlacing network that gives the web a dark appearance (Fig. 4). In the tree-toad, according to Schmidt (1920 b), there are, in addition to the melanophores, the so-called lipophores and guanophores through whose interaction the green, yellow, and grey tints of the toad are produced. In the fishes the combination of colour elements are often much more complex. Thus Connolly (1925) has shown that in *Fundulus*, the fish, beside changing from light to dark or the reverse, may assume with appropriate backgrounds a pink, yellow, or bluish tint. In addition to melanophores this fish possesses in its skin glistening bluish green bodies, the iridocytes, and yellow chromatophores or xanthopores, which constitute a system in many ways quite distinct from the melanophores (Fries, 1927). Much more complicated is the chromatophoral system in the flat-fishes. The fishes, whose colour changes have been extensively studied by Sumner (1911) and by Mast (1916), are truly remarkable. *Paralichthys*, according to Mast, is one of the most notable of these, for it can change not only from a very dark hue to almost white, but it can also assume tints of blue, green, yellow, orange, pink, or brown, a range quite equal to that of the chameleon. The physical basis for these changes, a basis that must be enormously complex in comparison with that in the simpler types, has been in part analysed by Kuntz



Fig. 4. Chromatophores from the web of the frog's foot. *a*, expanded, dark animal; *b*, intermediate condition; *c*, contracted, light animal. From Hogben, *The Pigmentary Effector System*, p. 3, Fig. 1, 1924.

(1917). Here, as in other chromatically active vertebrates, the melanophore is the essential element and the colour-play is brought about through its expansion and contraction in relation to other more or less fixed coloured bodies.

Among the reptiles as among the fishes there is a whole series of chromatophore systems from relatively simple types to most complex ones (Fig. 5). In *Phrynosoma* the whole range is from light to dark and the reverse (Parker, 1906), and is presumably based almost exclusively on melanophore activity. In *Anolis* active melanophores are associated with a quiescent leucophore layer and a layer of yellow oil droplets, and by means of these elements working in combination, the yellowish, emerald green, blue green, and mahogany tints of the living animal are produced (von Geldern, 1921). Schmidt (1918 b) has made a most exhaustive study of the chromatophores of the reptilian skin, and has identified four independent classes

of these bodies; these he has designated melanophores, guanophores, lipophores, and allophores. In the lizard *Feylinia* only melanophores are present. In other lizards various combinations occur which increase in complexity till such forms as the chameleon are reached, where all four kinds of chromatophores occur together. In consequence of this condition, the colour changes in the chameleon are perhaps the most remarkable of those in any animal.

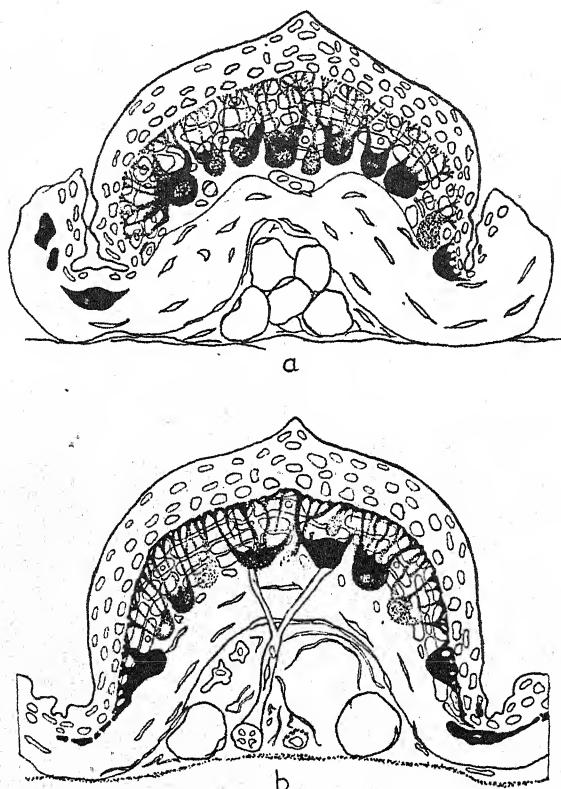


Fig. 5. Chromatophores in the scales of *Anolis*. a, melanophores contracted, animal green; b, melanophores expanded, animal brown. From von Geldern, *Proc. California Acad. Sci.* 4th ser. 10, Pl. 9, Figs. 11, 12, 1921.

Although the chromatophores of vertebrates show in their structure and activities many resemblances to those in crustaceans, they possess one striking peculiarity. In almost all instances the chromatophores of vertebrates when cellular in composition are single cells. They are very rarely complex in that they are syncytial in organisation, or in that they contain more than one class of pigment. In the great majority of cases each chromatophore possesses only a single kind of pigment, and that in a single cell. In only a few instances are there exceptions to this rule. Thus in some fishes, such as *Fundulus*, iridocytes composed of glistening, highly coloured materials that have an almost metallic lustre are found. These iridocytes

commonly occur in close association with the melanophores. Whether they are part of the melanophore, or a separate cell more or less imbedded in the melanophore, has not been determined. In certain respects they give rise to a condition of intermingled pigments such as commonly occurs in the crustacean chromatophore (Ballowitz, 1920).

Almost all cold-blooded vertebrates possess a more or less marked dermal colour pattern, and this pattern, as was long ago pointed out by van der Hoeven (1831), though intimately interwoven with the chromatophore system, is nevertheless in the main independent of it. The condition may be described as that of an underlying pattern of more or less permanent character, upon which has been superimposed a somewhat plastic and responsive system of chromatophores. Himmer (1923) has designated modifications of the underlying pattern as morphological colour changes and those of the active chromatophores as physiological colour changes. It is a matter of much importance in judging of the chromatic capacity of a given animal, to keep the two systems clearly in mind. Thus the lizard *Phrynosoma* possesses in white, grey, and black, a definite permanent pattern which is never absent, but which is accentuated or reduced by the active chromatophores. The same condition, except that it is much more complicated, occurs in the chameleon. In some cases, as, for instance, in *Anolis*, the permanent pattern is a most simple one and consists of little more than a dorsal dark coloration and a ventral light one, and in this instance as in the more complex cases, the chromatophoral system emphasises or reduces this pattern without obliterating it. In the flat fishes, however, the pattern is in large part involved in the chromatophoral system, and may vanish almost completely in certain phases of chromatophore activity. Thus in *Paralichthys*, according to Mast (1916), the whole upper surface of the fish may become quite white and thus lose almost all traces of the pattern that it usually shows. This obliteration of a colour pattern is, however, very exceptional. The transfer of a certain amount of the plastic chromatophoral or physiological activity to the permanent or morphological pattern of animals, and the establishment of this transfer as a part of the race heritage was one of the dreams of Kammerer. That this transfer to a certain degree can be made, seems clear from the work of Cunningham and MacMunn (1894), of Bábák (1913), of Himmer (1923), of Herbst and Ascher (1927), and of Herbst alone (1927).

Cunningham and MacMunn long ago showed that the illumination of the white underside of the flounder was followed by a development of pigment on that side. Bábák pointed out that expanded melanophores are more prone to division than contracted ones, hence any influence that will induce the expansion of such cells will increase their numbers, a conclusion accepted and supported by Himmer, Herbst, and Ascher. Thus, to take the fire salamander as an example, the retention of this animal on a black background will induce the expansion of melanophores and consequently their multiplication resulting in an increased darkening of the animal. Although this process transfers in a way a certain amount of the physiological colour change to the morphological system, it is extremely doubtful, contrary to the statements of Kammerer, if any of this transfer is ever incorporated into the inheritance of the stock.

The exact method by which a chromatophore, and especially a melanophore, appears to spread its pigment over a wide field or concentrates it into a small area, has never been accurately determined. According to Ruth and Gibson (1917) no real migration of pigment occurs in this process, but what is supposed to happen is the development of pigment from the centre outward and a reverse process of depigmentation from the periphery inward. These two operations produce what appears to be a migration, when in fact no such migration takes place. Essentially this view has been recently advocated by Murisier (1920) and by Kudô (1922). But so many observers have again and again followed, especially in fishes, the migration of individual melanin particles inward and outward, that it cannot be denied that in certain vertebrates at least migration does occur. Whether this process is supplemented by the formation of new pigment and the destruction of the old remains still to be proved.

The inward and outward migration of the pigment is so like the extension and the retraction of the pseudopodia of an amoeba that many workers have accepted what has been called the amoeboid theory of this movement. Ficalbi (1896) in fact believed that the vertebrate melanophore extended and retracted its pigmented processes exactly as the amoeba projects and withdraws its pseudopodia and with as much variability. But it has been shown by a number of workers, including for instance Spaeth (1913 *a*), that if a melanophore is photographed, and then made to contract, and to re-expand, a second photograph reproduces in full detail the branching system of the first. The inaccuracy of Ficalbi's opinion is therefore evident. The question in consequence has assumed a new form. Are there preformed branched spaces in the tissues around each melanophore into which its protoplasm and pigment may flow, draw back, and flow again, thus repeating its initial form (Fröhlich, 1910), or is the melanophore provided with permanent branched processes in and out of which the pigment streams, leaving the processes when empty so clear as to be almost invisible? Sharp and precise as this question is, its importance is perhaps slight, but, as is usual with questions of this kind, many workers have taken sides and much ink has been spilled in discussion.

In favour of a fixed outline within which pigment granules stream back and forth, an opinion held by perhaps the majority of investigators (Ballowitz, 1914; Kuntz, 1917; Dawson, 1920; von Geldern, 1921; Schmidt, 1921; Hewer, 1923), are the important considerations that the melanophore in its contracted state shows no delimiting outline appropriate to its new form, and that processes free of pigment can be identified. (Schmidt, 1921). Favourable to the amoeboid conception of melanophore activity, which was espoused some time ago by Hooker (1914 *a*, 1914 *c*), is the fact that after contraction, bits of pigmented cytoplasm are often seen as though left behind in the vacant spaces. It must also be remembered that melanophores in embryonic and larval fish exhibit great amoeboid activity (Stockard, 1915; Gilson, 1926 *a*) and thus show at this stage, in an indisputable way, that kind of activity that is attributed to them by those who advocate the amoeboid theory of action. It would not be surprising if chromatophores, and especially melanophores, should prove to possess in their early stages amoeboid activity and that this

activity with advancing maturity should gradually diminish so that in the end nothing would remain of it but the streaming in and out of pigment in a preformed process.

A nervous control of vertebrate chromatophores was assumed by most of the early workers, and this assumption was subsequently justified by investigations especially on the fishes. Ballowitz (1893 *a*, 1893 *b*) three and a half decades ago showed the richness of the nerve supply to the chromatophores of these animals, and von Frisch (1910, 1911 *b*, 1911 *c*, 1912) demonstrated by nerve cutting and other physiological steps that the fibres concerned were from the sympathetic system. Similar conclusions were reached by Schaefer (1921) on pleuronectids. Wyman (1924 *a*) showed that when a short transverse cut is made at the root of the tail in *Fundulus*, the nerve fibres radiating into a portion of the tail are intercepted, but the blood vessels are not. In the area of skin thus denervated the melanophores take on a condition of semi-expansion, the so-called stellate state, which they permanently retain, while those of the rest of the fish continue to undergo the usual expansion and contraction. These and other like tests have shown that the older investigators were correct in assuming that the melanophores of fishes at least could be controlled by nerves. Similar though less striking evidence of this kind has been advanced for the lizard *Phrynosoma* by Parker (1906) and by Redfield (1918), and very recently for the chameleon by Hogben and Mirvish (1928). Kahn (1922) has argued for a double innervation of the chromatophores in the frog, but Kropp (1927) has demonstrated that only a very limited nerve control may be assumed for this animal. This conclusion is quite in agreement with the recent work of Slome and Hogben (1928) on the toad *Xenopus*. May (1924) pointed out that when a piece of *Anolis* skin, rich in chromatophores, is transplanted from one spot to another on the animal or merely cut under in such a way as to sever possible nerve fibres, it ceases to show the usual colour changes. These, however, return after an interval long enough to allow for the regeneration of the nerves. May's results thus conform to what would be expected on the assumption that vertebrate melanophores are under nerve control. In general, the nervous control of melanophores, and possibly of other chromatophores, seems to be highly developed in fishes, less so in lizards and only very slightly so in amphibians.

Investigators are almost universally agreed that the light that enters the vertebrate eye is the most potent factor in controlling the colour changes in these animals. If one eye in a chromatically active vertebrate is covered or removed, the colour play may still go on, but if both eyes are incapacitated, it ordinarily ceases completely. This peculiarity has been repeatedly demonstrated in the past and is known to be characteristic not only of such simple colour reactions as those of *Fundulus*, the frog, and *Anolis*, but also of the complex ones, as, for instance, those in the flat-fishes and in the chameleon.

It is the rule in most vertebrates as in most other animals capable of colour change, that they become dark on a dark background, and light on a light one. This is accomplished through the eye by expansion of melanophores when the animal is on black and a contraction of them when it is on white. If such a reactive animal is

put in the dark, its chromatophores do not expand as might be expected but contract and thus produce a state of affairs ordinarily characteristic of a white background. This condition was first found in fishes by von Frisch (1911 b) and was subsequently confirmed by Parker and Lanchner (1922). It is known to occur in the frog and has already been pointed out for crustaceans. The effects of darkness and of a black background on the eye must therefore be regarded as quite different, for the types of chromatophoral response called forth by each are strikingly unlike. That *Salamandra maculosa* and *Phoxinus* are light-coloured by night and dark-coloured by day, as observed by Pauli (1926) may be additional examples of this type of reaction.

Light, however, may serve as a stimulus for the skin of vertebrates, as well as for their eyes. Laurens (1914 a, 1914 b, 1914 c, 1915, 1916, 1917) has repeatedly shown that colour changes are possible in the blinded larvae of salamanders. It is conceivable that this agent may act on the vertebrate skin, in one of two ways. Either it may stimulate the chromatophores of the skin directly or it may excite the dermal receptors and thus initiate reflexes which may bring about responses in these chromatophores. The direct action of light is well exemplified when it impinges in sufficient amounts on the human skin and induces the formation of pigment there, thus causing it to become brown. The indirect action may be seen in a frog from which the eyes have been removed. Such an animal is still responsive to light in that it will orient its body in reference to a given source (Parker, 1903) and thus show that the nervous mechanism for reflex movements in the animal can be excited through the skin. What is the condition in the vertebrate chromatophores? Do they respond directly to light thrown upon them, or is their response indirect in that they are the effectors in a reflex arc?

It appears to be quite certain that the melanophores of some vertebrates may be stimulated directly by light. If a piece of dorsal skin from the lizard *Anolis equestris* is cut from the animal and put in sunlight, it will turn brown, according to Hadley (1928), in 30 to 40 seconds, and if then it is returned to shade it will become green again in 12 to 15 seconds. These reversals back and forth may continue for as much as 3 hours after the skin has been prepared. What happens in sunlight can also be demonstrated by strong artificial light. If a piece of *Anolis* skin half an inch wide and two inches long is set out, one half of it in shadow and the other half in sunlight, the part in shadow quickly becomes green and that in sunlight quickly brown. If now the light and shadow are reversed on the skin, the colours reverse in about 1 minute. These observations show beyond a doubt that the pigment migrations in the melanophores of *Anolis equestris* may be induced by the direct action of light. A similar condition will probably be found in many other vertebrates, though how general it may prove to be is still to be discovered.

It can also be shown that the dermal receptors of certain vertebrates may be stimulated by light and that chromatophoral reflexes may thereby be initiated. Thus *Anolis carolinensis*, like other members of this genus, is mahogany brown in bright light, and bright green in the dark. If a single specimen of this lizard is put in a closely fitting light-proof form in which it cannot change its position to any

great extent, it will soon assume the green coloration. If now a minute hole is opened in such a form so that a small beam of light can be thrown on the flank of the lizard without, however, illuminating any other part of its body, the whole lizard in a short time will turn brown. This is very probably a nervous reflex, though it is conceivable that it might be due to a hormone. Redfield (1918) has described tests to the same end carried out on *Phrynosoma*, and these tests are not open to the objection just raised. By appropriate cuts, areas of skin on the flanks of a horned toad were prepared in which all nerve connections were severed. Nevertheless the melanophores of these areas expanded on local illumination and contracted when locally in the dark, thus showing evidence of direct stimulation. A confirmatory case has been reported by Schaefer (1921) for the flat-fishes. If a spot on the pigmented surface of a flat-fish is stimulated electrically, the whole body will become white. If now the spot is encircled by a cut so that the given piece of skin is connected to the animal only by muscle, local electrical stimulation whitens the spot itself but is no longer followed by general blanching. Thus the centripetal course in the original transmission must have been nervous. Now if the nerve supply to a portion of the pigmented skin is cut, thus denervating a small area, and the medulla is stimulated, colour changes may occur on any part of the body except the denervated part. Hence it is clear that the centrifugal transmission in this reaction is also nervous. What can be demonstrated by electrical stimulation very likely occurs in light stimulation, and it is fair to assume that light stimulates vertebrate melanophores directly as well as reflexly. It is worthy of note that in *Anolis* the eye and the skin work together; when one or both are in darkness the lizard is green, when one or both are in light the lizard is brown.

Heat, like light, acts upon the vertebrate chromatophores, especially the melanophores, causing them either to contract or to expand. In the great majority of cases, high temperature calls forth contraction, and low temperature expansion. Thus in the chameleon, to begin with reptiles, the contraction due to high temperature as claimed by the earlier workers has very recently been confirmed by Hogben and Mirvish (1928), and in *Phrynosoma* the contraction by heat and the expansion by cold as observed by Parker (1906) has been confirmed by Weese (1917) and by Redfield (1918). Among amphibians a considerable range of observations have led to the same conclusion. Thus contraction at high temperatures and expansion at low ones have been observed in the melanophores of *Ambystoma* larvae by Laurens (1915), of frog tadpoles by Cole (1922), and of mature frogs by Hogben and Winton (1923). The expansion of melanophores at low temperatures was noted in the frog by Hewer (1923) and in the newt *Diemyctylus* by Collins and Adolph (1926), but none of these workers observed a well-marked contraction at high temperatures. According to Dawson (1920) the melanophores of the perennibranch *Necturus* are contracted at low temperatures and expanded at high, just the reverse of the conditions reported for most amphibians.

The considerable uniformity noted in the responses of the melanophores of reptiles and amphibians to different degrees of heat is not to be found in the observations on fishes. A number of years ago Spaeth (1913 b) showed that the

melanophores in the isolated scales of *Fundulus* contracted at high temperatures and expanded at low ones, an observation agreeing with those in many of the older records. This, however, was in direct opposition to the observations of von Frisch (1911 a, 1912) whose carefully conducted experiments on *Phoxinus*, *Trigla*, and *Crenilabrus* had shown that the melanophores in those fishes expanded to heat and contracted to cold. This problem was reinvestigated in *Fundulus* by Smith (1928) who showed that the melanophores in the scales taken from the flanks of this fish reacted to heat by contraction and to cold by expansion as claimed by Spaeth, but that when they were left in place on the sides of the fish, as in von Frisch's experiment, they expanded to heat and contracted to cold. Searching tests on the part of Smith showed that the reaction of the melanophores in the isolated scale was due to the direct effect of heat or of cold, and that their reaction while they were in the sides of the animal, was due to a nervous reflex in which the temperature conditions stimulated nerve terminals in the skin, by which a reflex was initiated whose final outcome was a response of the melanophore. It is therefore believed that heat as a direct stimulus causes the melanophores of fishes to contract, and, as an exciter of nervous reflexes, causes them to expand. Cold acts in both respects in the reverse way to heat. From this standpoint the observations of most of the older workers, including Spaeth, on the reactions of fish melanophores to temperature, must be regarded as observations on melanophores directly stimulated and this conclusion very probably applies to the many uniform records on this subject for reptiles and for amphibians. The observations of von Frisch, on the other hand, give evidence of the responses of melanophores activated reflexly and with results just the reverse of those by direct stimulation. It is not impossible that Dawson's observations on *Necturus* belong under this head, for, as will be recalled from the statement in the last paragraph, his observations agree with those of von Frisch, and are the reverse of the statements made by the majority of workers for amphibians.

Not only are melanophores of vertebrates excited to action by temperature differences, but, according to Fries (1927), the yellow chromatophores or xanthophores of these animals are also thus brought into action. The denervated xanthophores in the tail of *Fundulus*, like the denervated melanophores of this fish, contract at high temperatures.

Since the chromatophores in vertebrates are bathed with lymph, it is not surprising to find that they are profoundly influenced in their activities by materials that may be dissolved in the fluids that surround them. The electrolytes of seawater, when appropriately applied, may be made to induce melanophore movements. Thus Spaeth (1913 b) pointed out some years ago that in *Fundulus*, sodium ions induce an expansion of the scale melanophores, and potassium ions the reverse. Many other electrolytes have been studied in this respect by Spaeth (1913 b, 1917) and by Lowe (1917). Uyeno (1922) has shown that oxygen accelerates and carbon dioxide retards melanophore contraction in the frog. The relation of various drugs to the movements of colour cells have been studied by a number of investigators (Lowe, 1917; Spaeth and Barbour, 1917; Spaeth, 1918; Wyman, 1924 a, 1924 b). Anaesthetics such as ether, chloroform, chloretone, novocaine, and cocaine (Wyman,

1922, 1924 b; Himmer, 1923) commonly induce an expansion of the melanophores and in the case of ether, this expansion has been shown to occur in denervated as well as in innervated cells; hence the action of the drug must be local though it may also influence the nervous system. Nicotine, according to Schaefer (1921) and to Wyman (1924 a), also calls forth expansion, though Hewer (1926 b) attributes to it a contracting influence.

Of the various materials that act upon the melanophores, the internal secretions have naturally excited much attention. Pineal extract, according to McCord and Allen (1917), will induce contraction in the melanophores of large tadpoles, but the extract that is most effective in this respect is adrenalin. The contracting effect of adrenalin of the frog melanophores was first described by Corona and Moroni (1898). Since the time of their publication, many workers have used this secretion, and with practically uniform results. Contraction of melanophores by adrenalin has been observed in such fishes as *Amiurus* (Bray, 1918), *Fundulus* (Spaeth and Barbour, 1917; Gilson, 1922; Wyman, 1922), *Phoxinus* (Albolin, 1924); and the flat-fishes (Schaefer, 1921; Hewer, 1926 b); in such amphibians as *Necturus* (Dawson, 1920), the frog, the toad and the salamander (Bigney, 1919; Hogben, 1924 b), and in the lizard *Phrynosoma* (Redfield, 1918). This contraction occurs in the denervated melanophores of *Fundulus* and hence the adrenalin must be assumed, at least in this instance, to act directly. As adrenalin is a natural product from the adrenal glands of vertebrates, it is not impossible that it is a material which as a hormone may be a normal means of controlling melanophore activities in the vertebrates (Redfield, 1918).

Another internal secretion of great importance is pituitrin. This substance has not yielded as uniform results as adrenalin. Thus Spaeth (1918) declared that it induced contraction in the scale melanophores of *Fundulus*, an opinion which has been supported by the observations of Hewer (1926 a) on the minnow, of Collins and Adolph (1926) on *Diemyctylus* and of Rowe (1928) on the frog. On the other hand, Allen (1917) showed that when the hypophysis of the frog is extirpated, the melanophores of the animal contract and remain thus with no apparent ability to expand. This observation was supported by the work of Smith (1916, 1920) and of Atwell (1919) on tadpoles. Albolin (1924) demonstrated that in the minnow, pituitrin induced an expansion of the melanophores. The problem took a very important turn in the hands of Hogben, who together with Winton undertook an extensive study of the action of pituitrin on the melanophores of amphibians (1922 a, 1922 b, 1922 c, 1923, 1924 a). This work was carried out on frogs, toads, and salamanders; and these investigators showed that the injection of pituitrin was always followed by a maximum expansion of the melanophores, that the excision of the pituitary body left the animal with the melanophore permanently contracted, that is, in continuous pallor, and, finally, that animals in this state could be made to expand their melanophores and thus become dark by the injection of foreign pituitrin. From these and other observations Hogben (1924 b) was led to conclude that pituitrin was a hormone of great significance in the normal darkening of such forms as the amphibians.

It must be evident from what has been stated in the preceding account of the

chromatophores in vertebrates, that the colour changes in these animals are, in the main, dependent upon the activities of their melanophores and further, that these melanophores, though more or less under the influence of external stimuli, are excited to action chiefly either through nerves or through hormones. In this respect the vertebrate chromatophores are in strong contrast with those of the cephalopods, which in their method of excitation are strictly nervous and with those in the crustaceans, which are excited in an equally exclusive way by hormones.

The approach to the chromatophoral system of the vertebrates, as to that of the cephalopods and the crustaceans, is almost exclusively through the eyes, though a photosensitive skin may play a minor part in this respect. Through the eye there may be excited reflex impulses for nervous control of the chromatophores, or for the secretion of hormones, which in turn may activate the chromatophores. When in this respect the three classes of cold-blooded vertebrates are compared, a considerable diversity is apparent. In fishes the nervous control appears to predominate, though as the experimental evidence shows, hormone control is not necessarily excluded. This view is supported by the fact that nerve terminals to chromatophores can be demonstrated with great ease in fishes. In amphibians the control seems to be almost entirely humoral with pituitrin as the expanding agent and adrenalin or some other like substance, as the contracting one. This conception of the amphibian chromatophore system as controlled almost exclusively by hormones, is distinctly novel and is due chiefly to Hogben and his associates. In the reptiles the two sets of influences, nervous and humoral, appear to be almost balanced, though not enough is known about the colour activities in this group of vertebrates to allow of final judgment.

VI. CONCLUSION.

The elementary effectors of animals consists of such parts as netting organs, cilia, luminous organs, glands, chromatophores, muscles, and electric organs. By means of these, singly or in combination, all animal responses are accomplished. In the firefly the flash of light, the odorous glandular secretion, and the directed muscular response are the elements out of which the immensely complicated sex life of this insect is made. Colour responses as mediated by chromatophores constitute no insignificant chapter in the book of animal habits. Notwithstanding the declarations of some of the older workers to the effect that mobile colour changes occur among sponges, sea-urchins and the like, it is improbable that these activities are found anywhere except among the higher animals. As the numerous surveys of this subject have shown, it is at least certain that chromatophoral activities are conspicuously developed only in the cephalopods, the crustaceans, and the cold-blooded vertebrates. All these animals have well-developed sense organs, especially eyes, and it appears that in all these groups the eye plays a most important part in initiating the chromatic change. This implies that the animal in a measure sees its environment and responds appropriately thereto. It is not to be supposed that the creature sees its surroundings and adjusts its own colour to fit. Even in some of the most complicated instances this does not seem to be the case. Thus in flat-fishes it can be shown that if a shield is placed between the fish's eyes and its body,

and the body is allowed to rest on a background of one tint while the eye sees another of very different tint, the body assumes the tint seen, and not that on which it rests. Thus chromatophoral responses, even in very highly organised instances, partake of the nature of a reflex rather than of higher nervous response. This elementary feature permeates the whole of chromatophoral activity and marks it as a reactive capacity of relatively low order. The colour changes of chromatophoral animals are never to be compared with the social blush of an old-fashioned belle.

Colour responses may be excited through the direct stimulation of chromatophores by external influences, through hormones or through nervous impulses. It has already been pointed out that the direct effect of the environment on chromatophores is relatively slight, and that they respond almost entirely through hormones or nervous activity. Hormones from their very nature permeate the whole animal and consequently call forth in it, colour changes that effect the animal as a whole. It is conceivable, however, that by blood-regulatory devices one part of the body might receive more of a particular hormone than another, and thus respond differently, but instances of this kind have as yet to be discovered. One hormone may, however, call forth different colour responses supposing the chromatophores to be differently constituted. Thus in *Palaemonetes*, according to the account given by Perkins, the hormone contraction produced by the eye stalk not only induces a contraction of the dark pigment cells, but an expansion of the pale yellow cells. This double response is in all probability to be attributed to a differentiation in the chromatophores, rather than to other causes. It recalls the well-known condition in vertebrates in which adrenalin induces a contraction in the dermal melanophores and an expansion in the retinal pigment cells. From this brief discussion it must be evident that hormones as a control of chromatophore movements, favour broad general responses and lack the capacity to call forth complicated and intricate patterns which are observable, to some degree at least, in many chromatic animals.

When the chromatophoral system is under nervous control, the independent action of different parts with consequent pattern formation is at once possible. Most colour patterns, as already pointed out, are, however, fixed features in an animal's organisation and the motile chromatophores are, so to speak, merely superimposed on such a system. But in the cephalopods and in the flat-fishes and possibly in such forms as the chameleons, colour patterns are chromatophoral possibilities. Thus both Sumner (1911) and Mast (1916) have shown that when especially responsive flat-fishes are placed upon chequerboard patterns of black and white, but of different degrees of coarseness, they respond by a coarser or a finer type of mottling appropriate to their surroundings. This approximate reproduction of a pattern is to be understood only on the basis of a récepteur and central nervous differentiation of a relatively high order, and indicates that in these fishes the chromatophores with their associated nervous equipment, are beginning to show a type of specialisation that was long ago undergone by muscle and its nervous connections, and that has reached in that system of effectors, a degree of development not approached by any other set of such organs. In some instances skin glands seem in this respect to be at much the same stage of development as the chromat-

phores of the flat-fishes. Thus the sweat glands of man usually function more or less as a unit, but they may and often do act differently in different parts of the body, thus producing a sweat pattern, so to speak, that is essentially similar to the motile colour pattern of a flat-fish.

The biological significance of the chromatophoral systems in animals is by no means obvious. No one, however, can observe the play of colours in a devil-fish or in a squid, or the agreement of tint between a shrimp and its environment, or the marvellous closeness of the coloration of a flat-fish to its background, without suspecting that these creatures are thereby closely adjusted to their environment. That, in this way, the young flat-fish can escape its foe, and the squid capture its prey, seems obvious to anyone who has studied these animals in their natural haunts. That protective and aggressive colorations, as indicated in the examples just mentioned, are functions of this system has been the belief of many workers (Stark, 1830; van Rynberk, 1906; von Frisch, 1912), an opinion still commonly held notwithstanding the opposition raised to it by such investigators as Schöndorff (1903) and Hess (1914).

Anyone who has observed closely the mating activities of chromatophoral animals, must often have been impressed with the height of the colour changes. In the male *Fundulus heteroclitus*, the maximum of colour effects characterise the breeding period. *Anolis* is also especially active in its colour reaction during breeding (Hadley, 1929). The males at this period are as a rule bright green, the females brown. When pairing takes place, the male is green for the first few minutes and then for the remainder of the period, an hour or more, brown. These colour changes have all the appearance of being part of the mating activity. Their significance is difficult to disclose, but that colour changes in such animals play a highly important part in breeding seems very probable. This emotional aspect of the subject has already been touched upon by Bauer (1914) and by Fuchs (1914).

That the changes from a light tone to a dark one and the reverse will alter the capacity of a given animal to absorb heat is self-evident, and that this change occurs with more or less appropriateness in many cold-blooded vertebrates, suggests that it may have functional significance. *Phrynosoma* is a semi-desert lizard whose daily rhythm or colour change, according to Redfield, is as follows: at night light-coloured, in the early morning dark, mid-day light, and in the late afternoon dark. These changes fall quite in line with what might be expected for thermoregulation, for early morning and late afternoon are the two times of day when the absorption of heat is possible and desirable. At these periods the lizard is dark. In the middle of the day when the heat may be excessive, the animal is light in colour. General colour changes of this kind are what have led a number of investigators to suspect that chromatophoral activity in certain animals might serve thermoregulation. This idea seems to have occurred first to Weber (1881) and was subsequently come upon independently by Krehl and Soetbeer (1899) and by Parker (1906). It has recently been elaborated by Bauer (1914), and by Krüger and Kern (1924). What value it has is yet to be determined, as may also be said of the opinion expressed by Keeble and Gamble, that in certain instances chromatophores have a photosynthetic function.

In conclusion it may be stated that the differentiated chromatophores of many animals appear to be concerned with protective and aggressive coloration, and that in special instances they may play an important part in the behaviour of the breeding season, in thermoregulation, and in other such subsidiary functions. But it must be kept in mind, as already stated by Mast (1916), that these views are one and all essentially hypothetical, and have never been really submitted to experimental test, that final test in scientific investigation.

VII. SUMMARY.

Chromatophores, the parts concerned with the colour changes in animals, are best developed in the cephalopods, the crustaceans, and the three lower classes of vertebrates, the fishes, amphibians, and reptiles. In the cephalopods chromatophores are really diminutive organs in that each one consists of a central coloured cell which is expanded against its own elasticity by radial muscle fibres. In the crustaceans a cellular complex often with several colours makes up each chromatophore. This expands and contracts slowly and thus changes the tint of the animal. In the vertebrates a coloured background in the skin is exposed to view or covered up by motile black pigment cells whose melanin granules are made to migrate within the containing cell. Most animals show relatively uniform changes of colour, but in a few, as, for instance, flat-fishes, a pattern may be imitated showing that the central control of the chromatophore system must be somewhat differentiated in the direction in which the musculature is.

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METHODS FOR THE PHOTO-ELECTRIC AND PHOTO-CHEMICAL MEASUREMENT OF DAYLIGHT¹

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(Received November 5, 1929.)

(With Seven Text-figures.)

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INTRODUCTION.

IN a vegetational survey of the globe one can see how the idea of the importance of temperature dominates all other considerations. This is partly due to the fact that temperature is susceptible of accurate measurement; in part the reason is anthropomorphic, and in addition there is a solid residuum of truth. In sharp contrast

¹ Being a paper read at the Agricultural Section of the Conference of Empire Meteorologists in London, August 29th, 1929, and printed in the Report of the Conference.

stands the meagre information as to the amount of light received in various latitudes and throughout the year. While we have the Callendar, Ångström and other radiation recorders to measure the total energy, no similar instrument has heretofore been available for measuring light alone.

Recourse has been had to various photo-chemical methods, and especially to photographic methods. A good summary of these has recently been given by Rübel (1928). A large number of measurements were made by Wiesner (1907) and his co-workers, using silver-chloride paper allowed to darken to a standard tint as originally worked out by Bunsen and Roscoe. These include measurements made in both tropical and northerly latitudes. Lundegårdh (1925) very properly considers light before all other factors in his discussion on plant distribution. He also points out that measurements made according to the Bunsen formula $S = i \cdot t$ (where S denotes the degree of darkening of the paper, i the intensity of illumination and t the time), are not correct, because the formula is not exact. This point has also been referred to by Klugh (1925), who considers that its neglect has invalidated all work done by this method. In view, however, of the great variations in light intensity found it seems unnecessary to condemn the careful comparative values thus obtained in so sweeping a manner. A direct comparison of the photographic paper and the photo-electric methods would undoubtedly be of value, especially as they both measure the violet end of the spectrum.

Reference may also be made to the classical researches of Abbot and his co-workers (1922) upon the distribution of energy in the solar spectrum at various places and at all seasons, and to the data obtained and collected by Dorno (1919). Measurements of illumination are also given by Walsh (1923) and in the works of Luckiesh (1915, 1922).

The advent of the photo-electric cell, more especially the vacuum type, has placed in our hands an instrument of a considerable degree of precision; this we have used since 1924 for daylight photometry. Its use permits of the calibration of photo-chemical methods also. An outline of both the methods and their results is given in the following pages.

OPTICAL CONSIDERATIONS.

Attention was called in some of our earlier papers (1925, p. 106, and 1926, 1, p. 180) to the optical conditions affecting the use of photometers whose windows consist of perfectly diffusing plane discs. In the general problem of measuring daylight account must also be taken of reflected light travelling upwards, an important factor over snow or water. In a recent paper (1929, 5) this has been discussed in some detail and is now given in an abbreviated form.

V denotes the vertical illumination on a horizontal plane, V_s , V_d and V_r being, respectively, the vertical illuminations due to pure sunlight, diffuse light from the sky, and reflected light that would be received on an inverted window small enough not to shade the ground below appreciably. Hence

$$V = V_s + V_d.$$

Also

$$H = H_s + H_d + H_r,$$

since the horizontal light falling on a vertical surface, unlike the vertical light on a horizontal surface, contains reflected light.

M is the illumination on a surface set to catch the maximum flux of light. In the open, in absence of sunlight, $M_d = V_d$. In sunlight the plane surface must be set nearly, but not quite, perpendicular to the sun's rays, the plane being always more nearly horizontal than the perpendicular, and markedly so when the sunlight is weak.

I , the total illumination, cannot be received directly on any single plane surface, but may be measured by a photometer whose absorbing surface is a complete sphere. It is defined as $\int_0^{4\pi} id\Omega$ where $id\Omega$ is the illumination due to a small pencil of rays of solid angle $d\Omega$ on a small area normal to the axis of the pencil. As before $I = I_s + I_d + I_r$, where I_s is the illumination from the angle comprising the sun's disc, $I_d = \int_0^{2\pi} id\Omega$ is that due to the sky, and I_r is the same integral over the lower hemisphere.

It is convenient to assume that both the sky and the lower hemisphere are uniformly bright all over. This is usually near enough to the truth for practical purposes, and implies *inter alia* that H_d and H_r are independent of the azimuth of the receiving surface.

The following relations hold, where α is the altitude of the sun, and H_s is the illumination on a vertical surface set in azimuth normal to the sun's rays:

$$(1) I_s = V_s \operatorname{cosec} \alpha = H_s \sec \alpha; (2) I_d = 2V_d = 4H_d, \text{ and } (3) I_r = 2V_r = 4H_r.$$

When a photo-sensitive liquid is exposed to light in a transparent tube, whose length, L , is great compared with its diameter, D , if the liquid absorbs strongly it may be considered as a cylindrical surface, instead of a plane surface as used in the photo-electric photometers. The illumination measured is then the mean for all directions normal to the axis of the cylinder, instead of that perpendicular to a plane. This must be remembered when comparing the results obtained with the two types of photometer, viz. the uranyl oxalate and methylene blue tubes compared with the photo-electric photometer.

Thus a vertically exposed tube measures the mean horizontal illumination for all azimuths, \bar{H} , the total light flux received being

$$\pi LD(H_d + H_r) + LDH_s, \text{ or } \pi LD\bar{H},$$

where

$$\bar{H} = H_d + H_r + \frac{H_s}{\pi}.$$

It is obvious that, when α is large, direct sunlight does not contribute very much to \bar{H} , as H_s (*i.e.* $I_s \cos \alpha$) is small. This must be remembered when interpreting results obtained in different latitudes.

In the absence of sunlight the orientation of an exposed tube is immaterial, so that the rate of reaction corresponds to a light flux

$$\pi LD(H_d + H_r), \text{ *i.e.* } \pi LD \left(\frac{V_d + V_r}{2} \right).$$

In direct sunlight the light flux or a horizontal tube will be increased by LDV_s (*i.e.* $LDI_s \sin \alpha$) when the axis of the tube is in the sun's azimuth, and by LDI_s when it is perpendicular to it. Thus we find that the mean vertical illumination recorded by the tube varies, according to its azimuth, from

$$\frac{V_d + V_r}{2} + \frac{V_s}{\pi} \text{ to } \frac{V_d + V_r}{2} + \frac{I_s}{\pi}.$$

When a tube is standardised against a lamp, as are Hill's methylene blue tubes, the effective area of the tube is only LD , whereas when used to measure \bar{H} its effective area is πLD . A tube standardised against a lamp therefore measures

$$\pi (H_d + H_r), \text{ i.e. } \frac{\pi}{4} (I_d + I_r),$$

as far as diffuse skylight and reflected light are concerned; for pure sunlight it measures H_s (*i.e.* $I_s \cos \alpha$), or I_s , according as the tubes are exposed vertically or normal to the sun's rays. Thus the fact that diffuse skylight produced more action, as found by Hill (1927), on such tubes than did the direct sunlight does not necessarily mean that the same would be true for a plane surface.

The measurement of I is probably the best way of recording the photosynthetic activity of the light for a unicellular alga, and was for that reason adopted in our 1925 paper. It requires, however, a knowledge of the angular distribution of the light unless a spherical surface is used. In all our later work with a plane receiving surface we record what is actually measured, V , or sometimes M , since the latter accords better with the distribution of plants in the purlieus of a wood.

Corrections must be introduced for reflection losses at the photometer windows; the value of this factor for pure sunlight only becomes 1.01 when $\alpha = 50^\circ$, 1.02 at 40° , 1.06 at 30° , 1.16 at 20° . For uniformly diffused skylight it is 1.06. Values of f and $\text{cosec } \alpha = \frac{I}{V}$ have been tabulated (1926, 1, p. 184 and 2, p. 280).

MEASUREMENT OF THE PHOTO-ELECTRIC CURRENT.

(1) *Galvanometer methods.* The current may be measured by a galvanometer, a portable instrument being required for work out of doors. For this purpose we have used a Tinsley portable galvanometer and an Onwood micro-ammeter (Gambrell) with suitable shunts as shown in Fig. 1.

(2) *Potentiometer null point method for use on a ship.* For work at sea we have used a potentiometer method (Poole 1925). In it the current is measured by noting the drop in potential across 100,000 ohms using a telephone as a null point indicator. An interrupter, made from a "Meccano" motor with slowing vanes, is included in the telephone circuit, which is shunted by a condenser, and the sensitivity is increased by the use of a two valve amplifier. Fig. 2 shows the general arrangement of the apparatus, the amplifier being attached to the back wall of the box, with high tension batteries on the side walls. The interrupter is visible in the middle, next the potentiometer, which is in front. In the left-hand lower corner is shown a gun-metal submarine photometer, and next to it one half of a deck photometer, mounted in gimbals, appears.

This arrangement was subsequently modified to secure better insulation in damp weather. Fig. 3 shows the present form.

The potentiometer being divided to 0.1 millivolt and the resistance being 100,000 ohms the current measured is 10^{-9} amp. per scale division. Under the best conditions readings may be made to 0.5 scale division.

(3) *Integration over a short period with the neon lamp.* The neon lamp photometer, introduced by J. H. J. Poole (1928) offers a method of measuring illumination

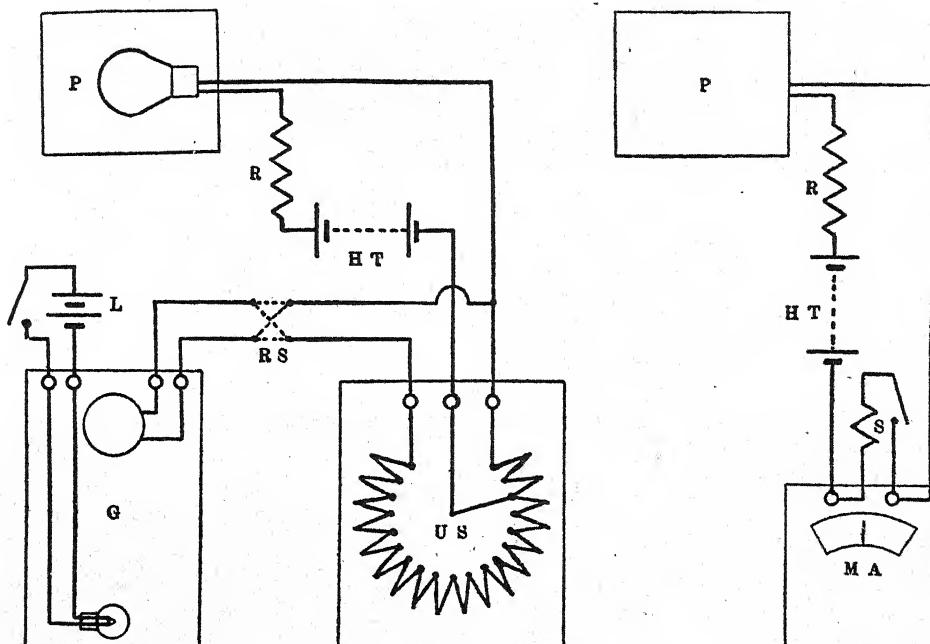


FIG. 1.

Arrangement of photometers and galvanometers at shaded base, left, and open base, right. L, galvanometer lamp battery. G, portable mirror galvanometer. RS, reversing switch. US, universal shunt. HT, high tension batteries, 60 volts. R, safety resistances, 10,000 ohms. P, photometers, the photo-electric cell is indicated in that on the left. MA, Onwood micro-ammeter. S, shunt, to one-fifth. For standardization of G, the P circuit was removed, and MA, with dry cell, connected to US instead. For comparison of the three photometers they were connected, in quick succession, to US, but only one is shown in figure.

which is sensitive, reasonably portable, robust, comparatively cheap, and has the great advantage that it integrates the illumination over the time of measurement, so that it should prove specially suitable for determining average values in sites subject to rapid fluctuations, such as just below the surface of the sea and in shallow water in rapid motion, or in a woodland site where swaying branches cut off sunlight intermittently.

If a small current, such as is obtained from a high tension battery through a very high resistance or a photo-electric cell, charges up a condenser across the

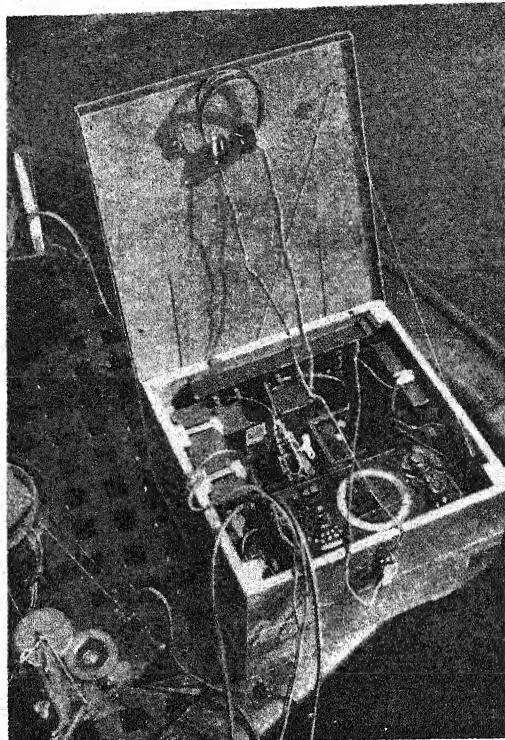


Fig. 2.

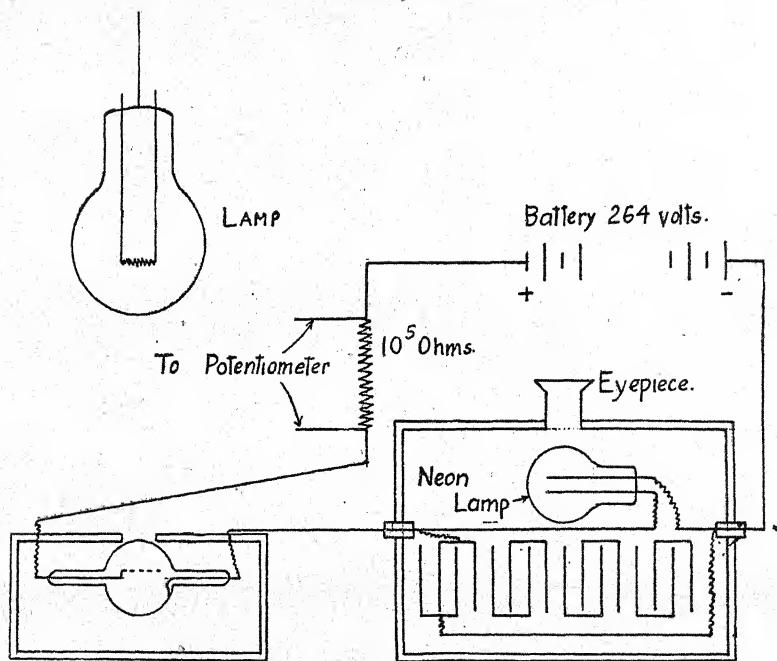


Photo-Electric Cell

Fig. 4.

Photometer

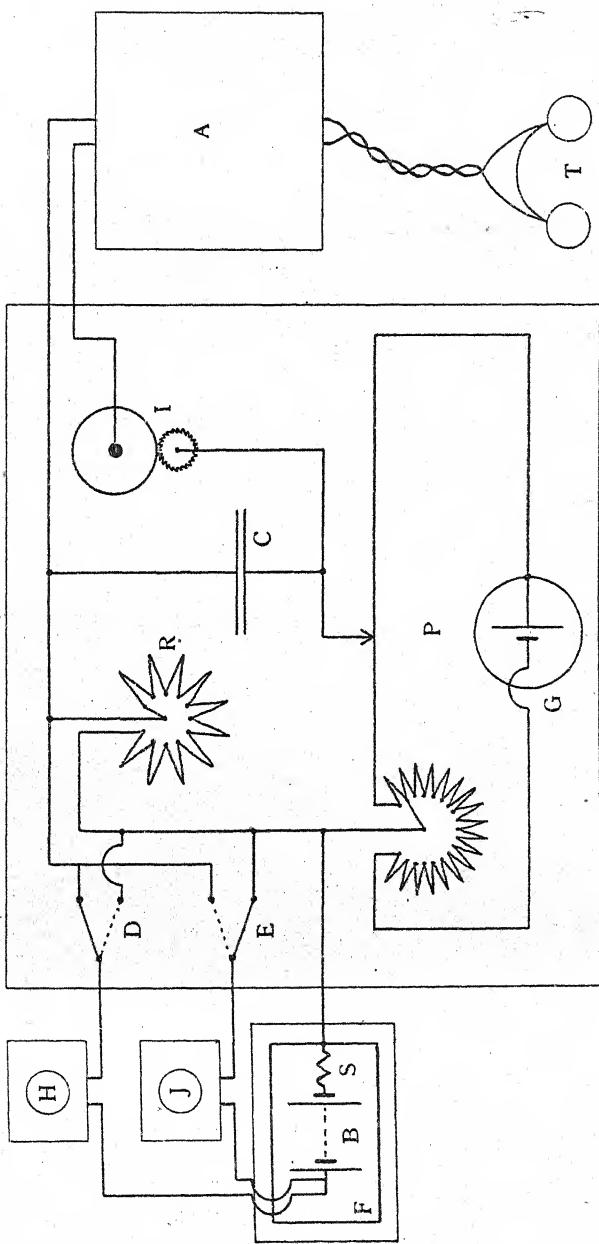


Fig. 3. *A*, amplifier. *B*, high tension batteries. *C*, condenser. *D* and *E*, switches. *F*, metal box, insulated from outer wooden one. *G*, guard plate, on which stands potentiometer accumulator. *H*, vacuum photo-electric cell in deck photometer. *J*, interrupter. *P*, potentiometer. *R*, 100,000 ohm resistance. *S*, 10,000 ohm safety resistance. *T*, telephone.

terminals of which a neon lamp is connected, the latter partially discharges the condenser at intervals, the discharges being rendered visible by flashes which occur at a rate which is a function of the charging current. Fig. 4 shows diagrammatically the arrangement used by Poole to ascertain the nature of this function, and Fig. 5

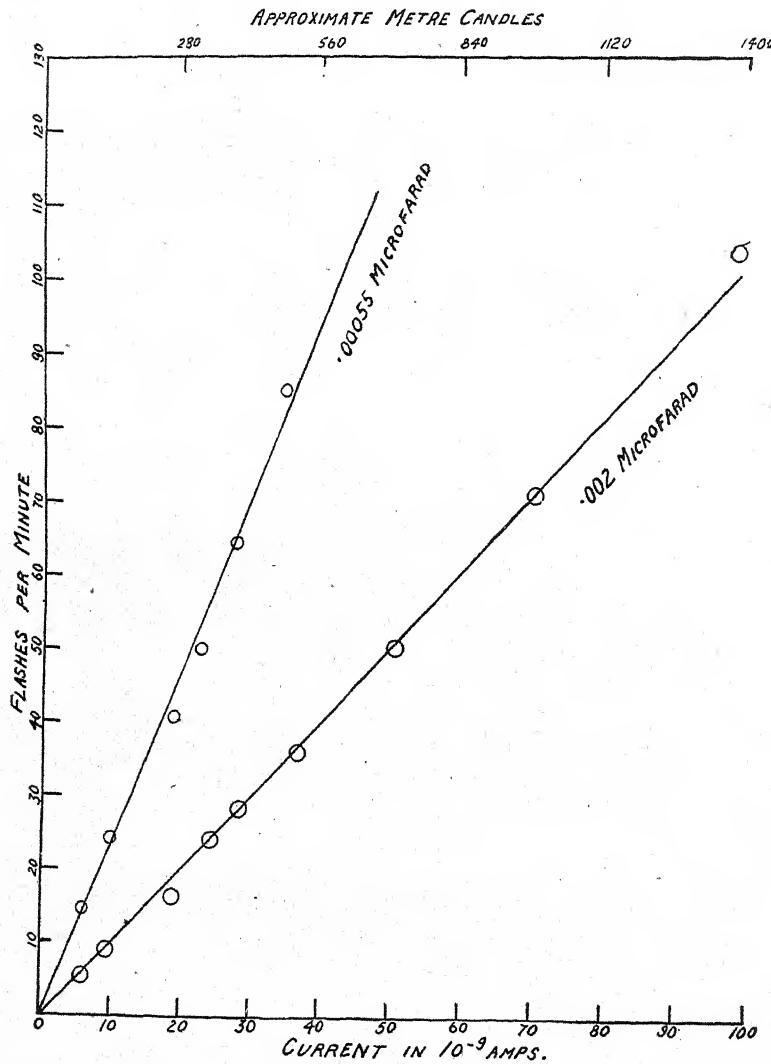


Fig. 5.

makes it clear that for each value of the capacity the relation between the rate of flashing, as measured with a stop-watch, and the current is a linear one.

The apparatus has since been improved by the addition of a system of guard rings and the use of a special type of neon lamp, which, by eliminating the effect of surface leaks, render the rate of flashing proportional to the current.

Accordingly a calibration of the apparatus, for each condenser, in terms of current or with a particular photo-electric cell in metre candles, enables one to determine the illumination by timing the rate of flashing. Using a vacuum sodium cell of the Burt type illuminations of less than 1 metre candle could be measured with a shunting capacity 0.0005 microfarad; by increasing the latter up to $0.5 \mu F$ the illumination, V , of full daylight could be measured. It is necessary to pay careful attention to the insulation of the neon lamp and condensers, of which a series (Dubilier type) are mounted in a box with the lamp. The only heavy and bulky part of the apparatus is the box of high tension dry batteries to give about 240 volts.

The flashing of the lamp may be demonstrated to an audience by the inclusion in the circuit of a two or three stage low-frequency amplifier and a loud speaker, when each flash is reproduced as a rap.

(4) *Integration over longer periods by photo-electrolysis.* For integration over longer periods use may be made of the method of photo-electrolysis (1929, 1). With a sensitive vacuum sodium photo-electric cell of the Burt type a very dilute solution of sodium bicarbonate may be electrolysed, the production of alkali being observed by the formation of a blue sheath round the cathode. On a bright afternoon in November this was noted within ten seconds after making the connection. With an illumination of 20,000 metre candles the photometer used, at 60 volts anode potential, would give 23 micro-amperes. This gives 55×10^{-6} mg. of sodium, or 2.4×10^{-6} mg. of hydrogen for the visible effect noted above in 10² seconds. Assuming the volume of the layer in which the blue colour was produced to be 0.1 c.c., in pure water the colour change would involve a diminution in hydrogen-ion content of 0.0095×10^{-6} mg., which is about 250 times less than the change involved in the experiment. Thus, by boiling out most of the carbon dioxide, and adding an even more minute amount of bicarbonate than was actually used, it would be possible considerably to increase the sensitiveness of the reaction. The method can be made quantitative with suitable precautions, but for use over periods of a day or more the photo-electric deposition of copper is preferable. It is advisable to use a copper anode, as the solution does not then become more acid, since the acid liberated attacks the anode instead of redissolving some of the metal deposited on the cathode. About 0.13 mg. of copper is deposited on a winter day, taking this as 10,000 metre candles for 10 hours. A summer day averaging 50,000 metre candles for 14 hours would deposit 0.92 mg. with the same sensitive cell. The estimation of the copper was carried out by the potassium ethyl xanthate method of Scott and Derby (1922).

(5) *Continuous measurement by means of a recording galvanometer.* It is obvious that the daily variations in illumination may also be followed, and the total quantity integrated, by recording the galvanometer deflections of method (1) on paper on a revolving drum. For this purpose one of the standard 25-hour drums is being modified, as, oddly enough, 24-hour drums do not appear to be stocked.

In measurements of this sort, as in routine photo-electrolysis, attention has to be paid to the absence of weathering of the exposed glass surface of the photometer

and to the constancy of the anode potential, as a source of which the use of storage batteries is to be preferred to the usual 60-volt dry battery (1930, 1).

PHOTO-ELECTRIC CELLS.

(a) *Types of cell, colour sensitivity and use of light filters.* A typical photo-electric cell consists of an evacuated bulb inside which has been deposited a film of an alkali metal, a window free from deposit having been left for the entrance of light. The film constitutes the cathode, the anode being a wire ring or grid placed between the window and the coated surface opposite it. A guard ring lead is attached

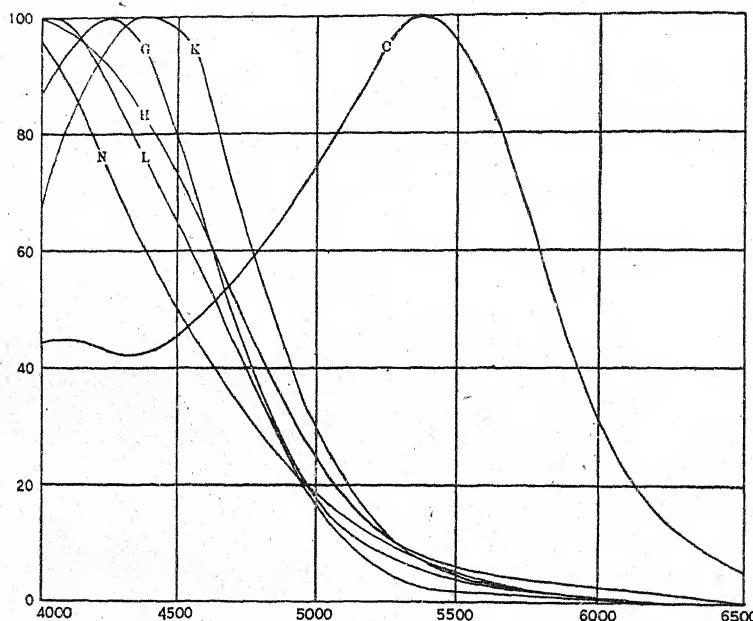


Fig. 6. The ordinates show the percentage sensitivity of the photometers for various wave-lengths as plotted in Ångström units on the abscissae. The photometers contain photo-electric cells as follows: C, a gas-filled caesium cell; K, a gas-filled potassium-hydride cell; G, H, and L, vacuum potassium cells; N, a vacuum sodium cell.

near the anode, but is only required, as a rule, for measurements of great sensitivity. The highly evacuated type of cell is stable and shows neither time-lag nor fatigue. It is said to be permanent even over long periods, and our experience confirms this. This type of cell was introduced by the General Electric Co. (1924). Those prepared by R. C. Burt are of necessity limited to sodium only, since the metal is deposited by electrolysis of the glass. These cells are of large size, like an electric light bulb, and are mounted on a radio-valve socket.

Highly sensitive cells filled with helium, argon, or neon under suitable low pressures have been in use for many years. The sensitivity may be still further increased by the formation of a colloidal hydride of the metal by making it the cathode in a glow discharge through hydrogen at a low pressure. The hydrogen

remaining is removed and replaced by an inert gas. Our experience with such cells has been that they lack the constancy of the vacuum type, and their use should be avoided whenever sufficient sensitivity can otherwise be obtained. The large Burt cells are very well suited for portable apparatus. Reference may be made to Allen's *Photo-electricity* (1925) for a general account of all types known up to that date.

The manner in which photo-electric activity varies with wave-length is somewhat complicated, and for a discussion of the factors concerned reference may be made to Allen. Even with the same alkali metal small variations are experienced from cell to cell. The colour sensitivities of various cells used by us are shown in Fig. 6. These were determined by measuring the energy in a spectrum band with a Moll vacuum thermopile and a very sensitive Kipp type Z galvanometer, the latter being also used to measure the photo-electric current. The ratio of the photo-electric to the thermo-electric current, for any given lamp setting, was taken as the relative sensitivity of the cell for light of wave-length corresponding to the centre of the spectrum band used (1928). These determinations were made with diffusing windows of ground glass in the various photometers. Since then a much better diffusing surface, glass opal-flashed on both surfaces, has been substituted. The importance of this may be recognised by inspection of Fig. 7. With the double surface flashed opal glass (J. Hetley and Co.) the theoretical sine curve, shown dotted in the figure, was obtained. This glass is, moreover, approximately non-selective in its action on light of various wave-lengths in the visible range.

The ultra-violet range is, however, heavily cut out by this glass. The effect is to render the sodium and potassium photo-electric cells very similar in their response to the ultra-violet when thus mounted, even though the Burt sodium cell is stated to have a maximum sensitivity at about 3600 Å.U. Unscreened, it measures the ultra-violet well; with clear sun and blue sky it gives 14 per cent. of its total unscreened reading when screened with a red purple Corex A filter, 10·2 mm. thick, which reduces the visible almost to nil and transmits about 42 per cent. at 3400 Å.U. and 14 per cent. at 3000 Å.U.

(b) *Standardisation of photo-electric photometers.* An open carbon arc lamp was used (1925, 1928). In the more recent work use was made of the results of Forrest (1913) and N. A. Allen (1921). The former has shown that the luminosity perpendicular to the face of the positive carbon is about 173 candle-power per sq. mm., and the latter found that under the conditions of his work the current was 0·746 ampere per sq. mm. Combining these two we get 232 candle-power per ampere in a direction perpendicular to the face of the positive carbon. Using such a light source it was found that with photometer *H*, which contains a small G.E.C. vacuum potassium cell, 40 metre candles gives 10^{-9} amperes with anode potential 60 volts. This cell is about four times as sensitive as a similar cell, *G*, which we used for several years for the calibration of a gas-filled potassium hydride cell of the Kunz type. With 3 volts anode potential the latter was about twice as sensitive as *G*, increasing to 35 times with 120 volts. Another potassium-hydride argon-filled cell made by the General Electric Co. (photometer *J*) was found to be 66

times as sensitive as H , viz. about 264 times as sensitive as G , with anode potential 88 volts. By raising the latter so as to produce a glow discharge, and then working with anode potential somewhat below that necessary to produce the discharge

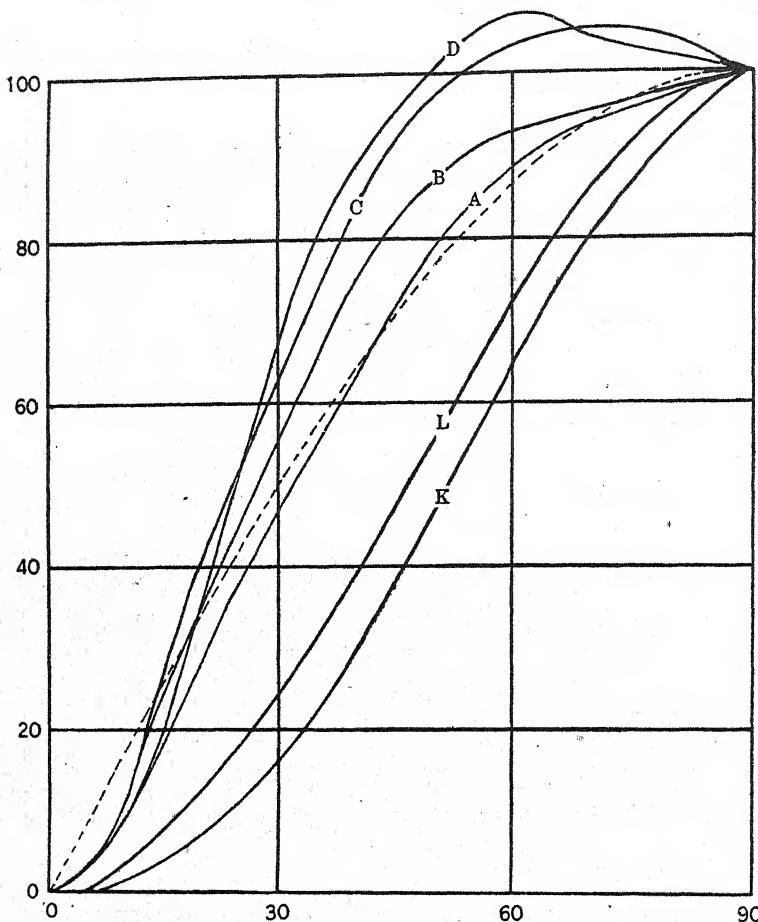


Fig. 7. The ordinates are percentage sensitivities of the photometers and the abscissae the altitude of the light source in degrees. Curves A , B , C , and D show the relative effects of a beam of parallel light of variable altitude for four rectangular azimuths on photometer G . Curves K and L show the mean effects for each of the four azimuths for these photometers. The differences between the various azimuths were very much smaller than with G , probably because the windows in the diffusing ground glass were narrower. The dotted sine curve shows what the effect would be with a perfect diffusing surface and no reflection losses at the front surface of the window.

with the illumination to be measured, both sensitiveness and constancy are greatly improved.

The Burt vacuum cells have been found to be from 19·4 to 50·3 times as sensitive as H .

(c) *Constancy of cells.* In order to satisfy ourselves as to the constancy of the vacuum photometers a test-lantern was constructed (1928).

It consists of a large white enamelled iron tumbler through the bottom of which a circular hole was bored to take a lamp-holder with a 6-volt, 18-watt vacuum lamp. The lantern was standardised at 5·50 volts for each photometer shortly after the latter had been standardised with the arc, and the illumination in metre candles recorded. As this lamp is only used occasionally for a few minutes, and at a reduced voltage, its candle power should remain approximately constant for years. No change has been detected in the photometers by its use, or by cross-checking them.

METHOD OF TAKING OBSERVATIONS.

When measuring the illumination in the open it is necessary to select a site as nearly as possible free from all obstruction to light, even of low angle. When at sea the stand, containing the gimbals on which the photometer rested, was lashed in position on the roof of the deck house of the Marine Biological Association's steam drifter-trawler *Salpa*, the stern of which was kept pointing towards the sun. This site afforded a very good view of the whole sky. On shore, positions such as the front parapet of the M.B.A. Laboratory roof, at Plymouth, an open position near the edge of the cliff at Cawsand, or the top of a garden wall on a hill at Antony were used. With the submarine apparatus, used at sea and in Cawsand wood (1926) both photometers are attached to one measuring instrument. This plan was also adopted at Antony in 1927 using a portable galvanometer, but in 1928 the use of cumbersome cable lengths was avoided by having the photometer in the open site connected to a micro-ammeter which was read at definite times by one observer, while another, using a portable galvanometer or a micro-ammeter with a more open scale, made the observations in selected shaded sites, noting the time of each.

RATIO OF TOTAL VERTICAL TO VERTICAL DIFFUSE LIGHT.

By shading the photometer window by the interposition of a small object held at some distance it is possible to cut off the direct rays of the sun without at the same time diminishing appreciably the light received from the blue sky or reflected from cloud masses. According to Aldrich (1922) the reflecting power of clouds is 78 per cent. $\pm 1\cdot1$ per cent., the value being independent of the zenith distance of the sun, but it seems likely it may vary with the type of cloud over a wide range. This ratio of the total to the diffuse vertical illumination may be denoted by β . Numerous measurements have been made using photometers *G* or *H*, both vacuum potassium cells. It was, therefore, of importance to ascertain whether, on changing over to the Burt type of cell for use with a micro-ammeter, any serious difference was introduced in the value of β . With diffuse light from a grey sky the ratio of the sensitivity of *B* 223 to *H* was found to be 50·3 on September 27th, 1928; on June 21st, 1929, with clear sun and blue sky, consecutive observations gave 51·8 for the same ratio, which appears to indicate that no appreciable changes had occurred in either cell, and that their ratio determined in white light from a grey sky and in a mixture of sunlight and skylight was closely the same, the sodium cell being perhaps slightly more sensitive to skylight. Thus on June 21st observations made in quick succession between 11.11 and 11.15 G.M.T. gave, with *H*, $\beta = 3\cdot62$

and 3.40, and with B 223, $\beta = 3.30$ and 3.21; the last value was obtained just before a cloud obscured the sun; at 11.17 the value was $\beta = 3.32$. On June 26th at 12.8 G.M.T., with B 223, $\beta = 3.52$; at 12.9, $\beta = 3.44$ with H , and at 12.11, $\beta = 3.47$ with B 223.

In general it may be said that $\beta = 1.5$ indicates weak "watery" sunlight, $\beta = 2$ may indicate sunlight at 3-4 p.m. in April with blue sky and heavy clouds or a hazy midday sky early in May with a few light clouds or similar conditions in October. Values such as $\beta = 3$ or 3.5 are found with light blue summer skies and a certain amount of diffuse light from clouds. Values such as $\beta = 4$ or over have been observed only when the vertical illumination exceeds 100,000 metre candles. On one occasion with bright sun, clear blue sky, and no clouds a value as high as 6 was found for β , as mentioned further on. The suspicion that too much skylight was cut off lurks in our minds, though it must be said that the pure sunlight, which is got by difference, was about what one would expect, instead of being much too high.

THE DAYLIGHT FACTOR, AND ITS DEGREE OF CONSTANCY: WOODLAND ILLUMINATION.

The best way of expressing the illumination in a wood, or shaded site, is, from the ecological standpoint, to use the daylight factor $\delta = \frac{V_w}{V_a}$, where V_w is the vertical illumination in the wood and V_a that in the open, neither receiving direct sunlight, and the illumination being taken as uniform over the whole sky. In practice the sky is rarely uniform, even in the absence of direct sunlight, so δ may be expected to vary somewhat. It is none the less, especially if averaged over a day, the best indication of the ecological lighting conditions, except at the edge of a wood, where the distribution of the vegetation is more in agreement with

$$\delta_m = \frac{M_w}{V_a}.$$

Table I. *Variations in δ .*

$\beta = 1.92$ at 1.15 p.m., October 13th, 1928, after that $\beta = 1.0$. $\delta = \frac{V_w}{V_a} \beta$.

G.M.T. p.m.	δ	Notes	G.M.T. p.m.	δ	Notes
1.15	55.8	Sun out, $\beta = 1.92$	2.26	66.2	Bright clouds in region of sun
2.19	56.0	Uniformly overcast	2.34	63.7	Disc not visible
2.20	57.0	—			
2.21	61.4	—			
2.22	61.4	Sky clearing at zenith			By uranyl oxalate, May 30th
2.24	61.4	—			a.m.
2.25	67.7	Zenithal blue patches	5.40-8.40 8.40-11.40	57.8 61.4	Overcast morning Murky, then rain and clearing up. Sun obscured throughout

Table I shows the variation found in δ on a garden lawn shaded to the south by trees and the north-west by a three-storey house.

If, however, it is desired to determine δ when the sun is shining use must be made of the factor β to correct the value of V and bring it to V_a , because the diffuse light in the wood is due to illumination coming from innumerable shafts of skylight; provided, of course, that no direct sunlight falls on the position of the photometer, then $\delta = \frac{V_w}{V_a} = \frac{V_w}{V} \beta$. Reasons based upon the percentage of the possible sunshine received during the six leafy months have been adduced (1926) to show that δ is also a good index of the general lighting conditions of a spot which receives direct sunlight for a portion of the day. Suppose a site has a daylight factor 75 per cent. and goes into shade at noon, β being 3 in the open, viz. total light 3, diffuse light 1, sunlight 2. The site will, therefore, receive up to noon a total illumination $2 + \frac{3}{4}$ and for it $\beta = \frac{2.75}{0.75} = 3.7$ (nearly). After noon the illumination will be $\frac{3}{4}$; so for the whole day the site will score 3.5 out of a possible 6, assuming continuous sunshine, also that $\beta = 3$ throughout, which is an over-estimate. On this hypothesis the site may be rated as receiving 58 per cent. of the possible. Since for England, S.W., the average number of hours of sunshine is 36 per cent. of the possible from May–October, the values may be weighted, and the site rated approximately as $\frac{58 \times 1 + 75 \times 2}{3} = 70$ per cent. almost, which is not very far from $\delta = 75$, especially since 58 per cent. is too low a value for the forenoon because β is below 3 for early morning and late afternoon sun. With a site facing north, for which $\delta = 50$ per cent., the use of the factor alone might be rather misleading, so the aspect should be stated.

ECOLOGICAL RELATIONS OF ILLUMINATION IN SHADED SITES.

It has been shown (1926) that in a mixed deciduous wood in early autumn δ may be as low as 1–2 per cent., which, it may be added, is about the value found at 20–30 metres in clear water off the coast of Cornwall, though the degree of clearness varies greatly. The floor of such a wood is devoid of grass, and is mainly covered with straggling *Hedera helix*, with perhaps a little *Lonicera periclymenum* and *Rubus fruticosus*, stunted and straggling on the ground with values $\delta = 2.9$ to 0.8, but more or less thriving with values from $\delta = 56$ to 16, and rather less than more with $\delta = 4.3$. Table II illustrates the method of studying such situations. The measurements were made with the submarine outfit, but the portable apparatus is now available (1929, 2 and 4).

The sites examined in Cawsand wood were as follows:

Site A. On path just below bank to east of wood, receives full sunlight up to about 11 a.m., but is shaded by wood. Mixed grasses and *Rubus fruticosus* flourish and received 61–52 per cent. of total daylight when examined, including some sunlight.

Site B. Under first tree *Fagus sylvatica* at edge of wood, a good N.E. aspect, *Quercus robur* scrub, *Acer pseudo-platanus*, young, *Castanea vulgaris*, young, *Pteris aquilina*, *Rubus fruticosus*, *Rosa arvensis*, *Bromus asper*, a little *Hedera helix*,

Geranium robertianum, *Lonicera periclymenum*; and, a little nearer the light, *Tamus communis* twining on *Pteris aquilina*; *Scilla nutans* frequent in spring; dry soil, pH 6.2, $\delta = 18$. About a metre nearer the light *Eupatorium cannabinum*, *Galium mollugo*, and *Dactylis glomerata* occurred. These three may require more light than $\delta = 18$, *Eupatorium cannabinum* previously found at $\delta = 34$ and *Galium mollugo* at $\delta = 16.5$.

Site C. Under second tree (*Fagus sylvatica*) in wood, 10 m. further in, much *Hedera helix* straggling on ground, also *Lonicera periclymenum* and stunted straggling *Rubus fruticosus*, some *Pteris aquilina*, *Fraxinus excelsior*, young, *Castanea vulgaris*, young, *Acer pseudo-platanus*, young, *Quercus robur*, young, *Rosa arvensis*, *Rubia peregrina*, *Lysimachia nemorum*, no grass; *Scilla nutans* frequent in spring; dry soil, pH 5.7, $\delta = 2.9$.

Site D. About 11 m. further in under young *Fagus sylvatica*, over-arched by other trees, *Hedera helix* straggling, dominant, a little stunted *Rubus fruticosus* and *Lonicera periclymenum* straggling, one plant *Primula veris*, dry soil, pH 5.8, $\delta = 1.8$. *Scilla nutans* frequent in spring, *Mercurialis perennis* absent.

Table II. September 10th, 1925. Area No. 2. Base in open, near edge of cliff, 20 metres east of Cawsand wood. All values of β refer to base. Photometers horizontal. Wind N.W.

Site	Weather conditions	G.M.T.	β	Illumination, metre candles		Percentages		
				In open V	In wood V_w	$\frac{V_w}{V}$	$\delta = \frac{V_w}{V} \beta$	pH
A	Sun through light clouds	11.13	—	33,800	20,600	61*	—	—
	Sun out, clear sky	11.19	—	74,700	38,900	52*	—	—
B	Sun out, clear sky	11.53	3.1	—	—	—	—	—
	Sun out, light fleecy clouds	11.55	—	81,500	4,770	5.8	18.1†	6.2
"	Clouds over sun	11.59	1.0	25,400	4,630	18.3	18.3	6.2
	Sun out, blue sky, light clouds	12.2	—	76,700	5,310	6.9	20.7‡	6.2
"	11.55 obs. with 11.59	—	3.2	—	—	—	—	—
	11.59 obs. with 12.2	—	3.0	—	—	—	—	—
C	Cloud over sun	12.42	1.0	25,400	730	2.9	2.9	5.7
	Sun through light clouds	12.45	—	54,100	830	1.5	3.1§	5.7
"	12.42 obs. with 12.45	—	2.1	—	—	—	—	—
	Cloud over sun, position of open base now shaded by wood when sun is out	2.5	1.0	34,400	620	1.8	1.8	5.8

* Received sunlight through topmost branches of wood.

† From $\beta = 3.1$.

‡ From $\beta = 3.0$.

§ From $\beta = 2.1$.

As well as an alteration in intensity there is, in a wood, an alteration in the spectral energy distribution. Klugh (1925) has discussed this; it had previously received attention from Zederbauer (1907) and the Burns (1924, 1927). By his photographic method Klugh showed that in dense spruce woods at St Andrews,

New Brunswick, on September 8th, 1924, with bright sun and a clear sky, the red, λ 5900 Å.U. and over, was 0.005 per cent. of the external red; the green, λ 4900–5900 approx., 0.05 per cent.; and the blue, λ 4000–5000 approx., 0.12 per cent. It seems obvious from these results that almost the whole of the light must have passed through plant tissues or been reflected by them. Our apparatus measures mainly the blue. The sites we examined being more open than the above, quoted from Klugh, the changes in spectral character were "diluted" by direct skylight. It is our intention to pay further attention to such alterations in the colour of the light in shaded sites.

SEASONAL CHANGES IN ILLUMINATION.

No time has as yet been available for making a regular series of observations, but Table III shows those made in the course of various researches. The maximum values for the vertical illumination vary from 134 to 11 thousand metre candles. Paterson and Walsh (1915) give values obtained in April–June and Sept.–Dec., 1914, for the diffused light on a white card shielded from direct sunlight, by means of an illuminometer.

The maximum value was 58,430 metre candles in June, for which month the mean was also a maximum, 44,130. Our maximum value for V_d is 69,500, for

Table III. Showing the maximum values of the vertical illumination in thousands of metre candles, recorded by a potassium photo-electric cell (standardised by means of a carbon arc) or sodium cell (standardised against a potassium cell) in open situations during the mid-day hours. β when determined on roof of deck house, at sea, is given to nearest 0.5, but when determined on shore is given to two places of decimals, with an error in the second, probably less than 0.05.

Date	β	V	V_d	Date	β	V	V_d
2. i. 29	1.0	15.2	15.2	7. ix. 27	4.0	100.0	25.0
1. iii. 28	4.0	66.5	16.6	9. ix. 27	1.0	33.9	33.9
6. iii. 28	3.0	75.0	25.0	10. ix. 25	3.1	81.5	26.2
27. iii. 28	1.5	66.5	44.3	12. ix. 27	1.5	65.2	43.5
5. iv. 28	2.5	86.0	34.4	18. ix. 28	2.5	78.5	31.4
19. iv. 28	4.0	134.0	33.5	23. ix. 25	2.8	58.9	21.0
7. v. 28	2.0	86.5	43.2	1. x. 25	1.5	24.5	16.3
13. vi. 28	1.0	69.5	69.5	2. x. 28	2.5	65.5	26.2
19. vi. 29	3.34	103.7	31.1	3. x. 27	4.0	73.3	18.3
20. vi. 29	1.00	30.6	30.6	5. x. 27	4.0	70.0	17.5
21. vi. 29	4.28	109.0	25.5	6. x. 27	2.0	45.3	22.6
26. vi. 29	3.47	103.0	29.7	12. x. 28	2.0	62.0	31.0
4. vii. 28	6.0	126.0	21.0	13. x. 28	1.92	54.5	28.4
11. vii. 28	3.0	130.5	43.5	2. xi. 28	2.19	46.9	21.4
23. vii. 28	4.5	121.5	27.0	3. xi. 28	1.0	17.0	17.0
9. viii. 28	1.5	56.0	37.3	9. xi. 28	1.61	35.9	22.3
29. viii. 28	4.0	111.5	27.9	30. xi. 28	1.0	15.3	15.3
1. ix. 25	1.0	54.2	54.2	14. xii. 27	1.5	30.8	20.5
2. ix. 25	3.5	84.4	24.1	17. xii. 27	1.0	13.0	13.0
2. ix. 27	4.0	109.5	27.4	19. xii. 27	1.0	12.5	12.5
3. ix. 25	3.0	74.0	24.7	20. xii. 27	1.0	11.1	11.1
5. ix. 27	1.0	48.9	48.9	21. xii. 27	1.7	33.5	19.7
7. ix. 25	3.1	73.6	23.7	22. xii. 27	1.0	11.0	11.0

June 13th, 1928, followed by 54,200 for September 1st, 1925, and 48,900 for September 5th, 1927—all three on days with sun totally obscured. With $\beta = 2$ on May 5th, 1928, $V_a = 43,200$, which was just surpassed with $\beta = 3$ on July 11th, 1928, when $V_a = 43,500$, with $V = 130,500$. With the maximum value found, $V = 134,000$, and $\beta = 4$, $V_a = 33,500$. Our table has twelve September records for V_a , which average 32,000, which is in good accord with 32,600, the value found by Paterson and Walsh. The important point is not the fortuitous closeness of the figures but the fact that the two totally different methods are in tolerably good agreement. A direct comparison is to be desired.

SUBMARINE ILLUMINATION.

Shelford and Gail (1922) introduced the photo-electric cell for measurements of submarine illumination and spectrophotometric measurements by Knudsen were published the same year. These and earlier photographic work have been reviewed by one of us (Atkins, 1926). We have elsewhere (1929, 3) described photo-electric measurements made in the English Channel throughout the year.

The vertical absorption coefficient, μ_v , measures the relative reduction of illumination per metre depth, μ = the true absorption coefficient depending upon the angular distribution of the light, which travels a longer course than d , the depth. It is, for vertical illumination, defined by the well-known equation $V = V_0 e^{-\mu d}$, whence $\mu_v = \frac{2.3}{d'} (\log_{10} p_1 - \log_{10} p_2)$ where the illumination p is recorded as a percentage of the simultaneous value of the surface light V and d' is the difference in depth between the two points.

Observations were made at the International Hydrographic Station, England, No. 1, E 1, 22 miles S.W. of Plymouth. These may be illustrated by the records of Series 28, shown in Table IV, made on April 19th, 1928, when the water was exceptionally clear. The illuminations are recorded in thousands of metre candles (k.m.c.) in air V and in water V_s .

URANYL OXALATE PHOTOMETRY.

Of the various photochemical reactions known and listed by Allmand (1925) and Kistiakowsky (1928) none appears to have greater advantages on the score of reliability and ease of manipulation than the decomposition of oxalic acid in presence of a uranium salt as catalyst.

It has been studied by many workers from Niepce de Saint-Victor and Corvisart (1859) onwards, notably by Bacon (1907, 1910), Büchi (1924), and also Anderson and Robinson (1925). We have reviewed earlier work elsewhere (1929, 5), and carried out a comparison between the rate of decomposition of the oxalic acid, exposed in quartz or glass tubes, and the vertical illumination determined photo-electrically.

Use has very generally been made of the proportions of the reagents given by Anderson and Robinson, namely, 0.1 N oxalic acid and approximately 0.01 M uranyl sulphate, $\text{UO}_2\text{SO}_4 \cdot 3\text{H}_2\text{O}$, viz., 4.27 g. per litre (0.01 M = 4.203 g.).

Table IV. Measurements of submarine illumination.

Date, remarks, etc.	G.M.T.	α°	Light	β	d metres	V k.m.c.	V_s k.m.c.	$\frac{p}{\%}$	μ_0
Series 28. 19. iv. 28. K photometer, at E. Wind N.W., light. Slight swell from N.W. Clear sky with clouds. High water 5.23 p.m. Secchi disc 18 m. at 4.18 p.m.	11.58 a.m. 12.8 p.m. 12.21 12.31 12.40 12.50 12.56 1.49 1.54 1.58 2.3 2.11 2.23 2.30 2.45 3.0 3.11 3.15 3.19 3.35 3.53 3.58 4.1 4.8	51 51 51 51 51 51 51 48 47 47 46 45 43 42 40 38 37 36 35 34 31 30 29 28	Bright sun Light cloud Sun Bright sun ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, ,, Cloudy Bright sun ,,	4 4 2 2 3.5 4 4 4 4 4 30 4 4 4 4 4 4 4 4 4 4 3.5 3.5 3 3 3	r a 5 10 10 15 15 15 20 25 30 35 40 45 50 55 60 65 70 45 25 25 r	109 114 45.5 49 81 121 134 102 100 100 99 97 94 94 89 84 79.5 78.5 77.5 74 73.5 25.3 69.5 68	109 109 22.7 17.2 26.7 41 29.6 24.6 15.6 10.4 7.35 4.15 2.12 1.20 1.28 0.65 0.342 0.192 0.116 0.148 0.076 0.73 5.8 1.77 5.55 68	100 95.5 50 35.1 33.0 33.8 22.1 24.0 15.6 10.4 7.4 4.25 2.25 1.28 0.725 0.405 0.242 0.100 0.148 0.098 0.985 7.9 7.0 8.0 100	— — 0.090* 0.077 0.077 0.077 0.078 0.078 0.079 0.075 0.088 0.119 0.121 0.114 0.115 0.109 0.100 0.090

* The asterisk denotes that the surface reflection loss was taken as 15 per cent. in calculating this value. a° = sun's altitude.

"r." photometer on rail in stern; "a." photometer swinging just above water.

The method was proposed by them for the study of lamps, such as the quartz mercury arc. For this purpose it has been used by Gillam and Morton (1927, 1928) and by us (1920, 6).

The most exhaustive measurements of daylight by this method are those of Tonney, Somers, and Marti (1928), whose procedure was to place 25 c.c. of the reaction mixture in a rectangular quartz cell $3\cdot35 \times 10$ cm., $1\cdot65$ cm. in cross section; the walls were all covered with opaque paper, save for an area of 10 sq. cm. on the lower part of one face through which the exposure was made. The vessel was placed so that the open face was always at right angles to the sun's rays. Exposures were usually made for one hour, the position being adjusted every 15 minutes. A large number of determinations were made from 1926 to 1928, and curves were obtained for the daily, monthly, and seasonal variations. The readings were correlated with the production of erythema on human skin, which corresponded to the destruction of between 3.52 and 3.72 mg. oxalic acid per sq. cm. during the time of exposure. The minimum time required to produce erythema was 35 minutes and other exposures up to 60 minutes, which just produced a definite erythema, showed a destruction of oxalic acid within the limits given. In all cases the skin had been sponged with ether to remove the natural oils, and the close agreement between the various subjects was probably due to this precaution. The maximum rate observed away from all smoke on the Indiana sand dunes,

30 miles from Chicago, was 7.12 mg. of oxalic acid per sq. cm. per hour. This is termed the climatic maximum. The authors consider their chemical results to be measurements of solar ultra-violet light, relying on the statement of Anderson and Robinson that absorption is complete up to 3600 Å.U. and partial as far as 4100 Å.U. According to Büchi, however, absorption proceeds considerably further into the visible spectrum; this is most important, as the solar spectrum energy distribution curve has a maximum about 5000 Å.U., and falls off very sharply towards the violet and ultra-violet. By making identical exposures in tubes of quartz and of Monax resistance glass, 0.9–1.1 mm. thick, we found the ration $Q/M = 1.14$, viz. the portion stopped by the Monax glass is only one-eighth of the total, yet it stops all erythema production, and cuts down the action on Hill's methylene blue tubes to under 40 per cent.

We prefer, therefore, to consider measurements of daylight carried out by uranyl oxalate as recording the blue, the violet, and, to a lesser extent or not at all, the long and the short ultra-violet.

When there is a grey sky one still gets a good destruction of oxalic acid, even though examination fails to show any ultra-violet above the intensity that can be detected with the Beck fluorescent ultra-violet spectrometer directed towards the sun's disc, visible through cloud. With a clear sun, however, when the radiation is above a certain intensity the ultra-violet begins to be effective and joins in the destruction of the oxalic acid. We have also used this method for determining the daylight factor of shaded sites for ecological purposes (1929, 4 and 5). For convenience in transport, and to lessen cost, quartz tubes were used, even though the optical considerations are thereby rendered less simple than when a plane surface is exposed, as has been pointed out in an earlier section. The tubes were 13 × 1.6 cm., internal diameter 1.3 cm. For general ecological purposes they may be replaced by similar ones of Monax glass. The quartz was used at first because of the mistaken idea that the glass was insufficiently transparent. Determinations were made using 10 c.c. of the reagent, and the tubes were exposed either on shore at 15° to the horizontal or at sea, swinging about a mean vertical position, to measure the horizontal light. They were also used to measure the submarine illumination, for which purpose they are less satisfactory than the photo-electric cell (1930, 2).

After introducing suitable corrections for the difference between the light received by the tubes and by the photo-electric photometer used for standardising, it was found that the N/10 oxalic acid solution was decomposed at the rate of 0.20–0.25 c.c., or 1.26–1.57 mg. per hour per thousand metre candles; the illuminations were measured by a potassium photo-electric cell, exposed beside the tubes on the laboratory roof, the latter being at 15° to the horizontal—for which a correction was introduced. The greatest value of the ratio was with afternoon sunlight, the least with a high noon sun, intermediate values being obtained with a grey sky.

In future we hope to make measurements of total intensity, I , exposing the solution in a spherical flask as suggested in the section on "Optical Considerations."

ULTRA-VIOLET PHOTOMETRY BY AN ACETONE
SOLUTION OF METHYLENE BLUE.

This method, though not capable of such accurate comparison, is a much better indication of the intensity of the middle (physiologically active) ultra-violet than is the uranyl oxalate photometer, since various glass tubes, superposed on the quartz, cut down the reaction to less than 40 per cent. according to our measurements, whereas the uranyl oxalate reaction, in a glass tube, proceeds at seven-eighths of its rate in quartz. For details Hill's (1927) paper is readily accessible for consultation. Attention should, however, be paid to the optical considerations as pointed out in an earlier section. Moreover, when the reaction has proceeded so that it corresponds to anything below 7, viz. three units of bleaching (its colour scale being 10 to 3), it is reversible, and recovers to a value close to 7 on standing overnight in the dark. Furthermore, with a tube laid horizontally, the apical end remote from the air bleaches more rapidly than does the general body of the tube beyond the middle point.

In conclusion, we wish to record our indebtedness to the Royal Dublin Society, and to the Marine Biological Association of the United Kingdom, for the loan of blocks and for permission to make use of material from their publications.

SUMMARY.

The optical conditions relating to plane, cylindrical, and spherical photometers are discussed.

A brief account is given of some galvanometers found to be suitable for the measurement of the photo-electric current in the field, of the potentiometer method for use at sea, and of the use of a recording galvanometer for continuous measurement of the variations of daylight. The current may be integrated over a short period by timing the flashes of a neon lamp shunted by a condenser, or, over longer periods, by the electrolytic deposition of copper.

Types of photo-electric cells are discussed in relation to their spectral sensitivity, constancy and standardisation.

Examples are given of photo-electric measurements of daylight in the open, in buildings, in woods, and down to 70 metres depth in the open sea.

A brief description is given of photo-chemical photometry by means of uranyl oxalate solutions and its standardisation against photo-electric measurements. The methylene blue method of ultra-violet photometry is also mentioned.

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SUR L'HISTOPHYSIOLOGIE DES ANNEXES FÉTALES DES MAMMIFÈRES

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(Received December 11, 1929.)

(With Four Text-Figures.)

L'ÉTUDE des annexes fœtales des Mammifères se présente comme l'une des plus complexes, car s'y enchevêtrent à la fois des problèmes morphologiques et physiologiques. Les premiers sont loin encore d'être tous résolus; mais de leur étude, poussée très loin, se dégagent des conclusions générales.

Il n'en est pas de même des seconds, beaucoup plus ardu. Parmi ceux-ci figure, en première ligne, la physiologie placentaire: débrouiller les relations qui unissent la morphologie de cet organe si varié à sa physiologie; et chez une même espèce, connaître son mode de fonctionnement intime: ce sont là des questions auxquelles morphologistes et physiologistes se sont attelés depuis longtemps, en employant des méthodes bien différentes. Les premiers, surtout observateurs, tiraient, de la constatation des rapports placentaires observés entre la mère et l'embryon, des déductions logiques, mais sans grande portée; les physiologistes essayaient d'aborder la problème par la voie expérimentale, en tâtant la perméabilité placentaire. Les méthodes physiologiques étaient basées sur le principe suivant: si l'on injecte une certaine substance à la mère, et qu'on la retrouve ensuite dans les humeurs de l'embryon, cette substance a passé au niveau du placenta. Conclusion trop hâtive, ainsi que nous le verrons tantôt, car elle postulait que la région placentaire est seule perméable aux substances contenues dans le sang maternel. Entre ces deux méthodes si opposées, en existe une autre, la méthode histophysiologique qui procède de toutes deux. Ce sont principalement les résultats obtenus par elle que nous exposerons ici. Cette méthode nous montrera également qu'une autre annexe fœtale, la vésicule ombilicale, intervient parfois, elle aussi, dans l'absorption des matériaux nutritifs. Nous ne parlerons ni de l'amnios, ni de l'allantoïde, leur histophysiologie étant encore à peine ébauchée.

Dans les pages qui vont suivre, nous envisagerons seulement les annexes fœtales des Mammifères placentaires, les seules sur qui ait porté quelque peu l'expérimentation, et en passant, nous ne manquerons pas de signaler les lacunes les plus importantes dans nos connaissances.

Le terme de Placenta s'applique, chez les Mammifères, au dispositif assurant les échanges entre la mère et les embryons qu'elle renferme. Ce dispositif

peut se présenter sous trois formes distinctes, mais non tranchées, car elles présentent entre elles des transitions multiples¹.

La première est représentée par le placenta épithélio-chorial, dans lequel le chorion fœtal s'applique intimement, sans effraction, sur la muqueuse utérine maternelle hypertrophiée en totalité; entre sang maternel et fœtal se dresse la sextuple barrière formée par les deux endothéliums vasculaires, les deux tissus conjonctifs, et les deux épithéliums, utérin et chorial. La seconde forme est représentée par le placenta endothélio-chorial, où le chorion fœtal, subissant une hypertrophie locale, ronge à ce niveau l'épithélium utérin, pour venir coiffer les capillaires maternels réduits typiquement à leur paroi endothéliale: c'est le placenta proprement dit. Le reste du chorion—segment paraplacentaire—constitue avec l'épithélium utérin sur lequel il s'applique, une formation du type placenta épithélio-chorial.

Dans la troisième forme se rangent les placentas hémochoriaux, où typiquement, le chorion fœtal prolifère en un endroit localisé, digérant les tissus maternels, y compris l'endothélium des capillaires: le sang maternel extravasé vient alors le baigner librement. Dans certaines formes, le chorion tout entier possède ce pouvoir lytique et pénètre par toute sa surface dans les tissus maternels: mais même dans ce cas, la prolifération et le pouvoir lytique du chorion se localisent assez rapidement en un endroit limité; celui-ci prolifère et s'organise de manière à drainer vers lui la plus grande partie du sang maternel, qui vient baigner directement ses villosités. Ici également se différencie donc, aux dépens du chorion, une partie placentaire, et une partie paraplacentaire: cette dernière, après avoir joué un rôle actif dans les premiers stades de l'implantation de l'œuf, voit son importance régresser rapidement; parfois même, cette régression va jusqu'à sa disparition complète.

De ces données fort succinctes, on peut déjà conclure à la diversité de la physiologie des échanges dans ces trois classes de placentas. Dans les placentas épithélio-choriaux, l'épithélium utérin se dresse comme une barrière entre le chorion fœtal absorbant, et les humeurs maternelles (sang et lymph). Nous ne sommes pas exactement fixés sur son rôle exact: joue-t-il le simple rôle de filtre sélectif laissant passer dans la cavité utérine certaines substances dissoutes dans le plasma maternel? ou bien fonctionne-t-il comme une véritable glande qui par un processus sécrétoire, c'est-à-dire de synthèse, élabore les matériaux nutritifs, puis les déverse dans la cavité utérine, où ils forment l'embryotrophe? Nous ignorons presque tout de la composition chimique de cet embryotrophe², complexe formé par la sécrétion de l'épithélium utérin, et celle de ses glandes: tout porte à croire que ces deux produits sont différents, mais nous ne pouvons préciser en quoi: entr'autres, nous ne savons sous quelle forme ni par lequel des deux épithéliums est livré à l'embryon le fer, qui lui servira à édifier son hémoglobine.

Nous connaissons un peu mieux l'histophysiologie des placentas endothélio-choriaux. Nous savons depuis longtemps qu'à la limite des deux parties du chorion, la partie placentaire et la paraplacentaire, existe une zone étroite, où du sang

¹ Nous adopterons la classification de Grosser (14) qui indique si clairement ces rapports et ferons abstraction des placentas syndesmochoriaux, qui font la transition entre les épithélio- et les endothélio-choriaux.

² Cf. Wertheimer (18).

maternel s'extravase entre le chorion et la muqueuse utérine; en cet endroit strictement localisé, les cellules du chorion phagocytent les hématies maternelles, les digèrent, en retiennent le complexe ferrique qu'elles cèdent à l'embryon, et rejettent la partie à noyau tétrapyrholique: on retrouve celle-ci accumulée, sous forme d'une bordure étroite périplacentaire qui, chez le chien par exemple, est d'un beau vert sombre; cette couleur est dûe à la bilirubine qui s'y accumule. Dans la région placentaire sont absorbées des substances ayant une double origine: 1^o la sécrétion provenant des glandes utérines hypertrophiées qui fonctionnent pendant toute la gestation et viennent se déverser contre le chorion (substances histiotrophes); 2^o des matériaux issus du sang maternel. Quelle est la part de ces deux formations dans la nutrition de l'embryon: en dehors de l'oxygène, le sang maternel livre-t-il d'autres substances à l'ectoblaste chorial?

C'est ce que certaines expériences ont tenté de préciser; telles par exemple, celles de Cunningham^(5, 6) vérifiées par Wislocki^(19, 20). Elles consistent à injecter dans la circulation maternelle soit du trypanblau, soit certains sels de fer. Ces substances s'accumulent, en se fixant, dans certains tissus où elles se mettent en évidence soit d'elles-mêmes (trypanblau) soit après usage d'un réactif révélateur (sels de fer); on peut ainsi les suivre à la trace dans leurs pérégrinations.

Certes, les expériences réalisées de la sorte sont toujours possibles de l'objection générale, formulée à chaque occasion de leur emploi, que ce sont des substances extraphysiologiques, et que le comportement des tissus envers elles est différent de ce qu'il est pour des substances physiologiques. L'argument, en soi, a une valeur évidente; mais cette valeur diminue si l'on songe: 1^o, que les substances physiologiques sont de celles que l'on ne peut mettre en évidence par les méthodes histochimiques, encore trop imparfaites; 2^o, que ce que l'on veut surtout étudier par ce moyen, ce sont les voies de sortie maternelle et d'entrée foetale: il importe en effet de déblayer le terrain, en précisant qu'il existe des voies électives pour le cheminement de certaines substances et que ces voies ne sont pas les mêmes pour toutes les espèces. C'est ainsi que si l'on injecte à des chattes en gestation un mélange balancé de solutions de ferrocyanure de soude et de citrate de fer ammoniacal—sels également diffusibles—le premier sel passe à travers le placenta et se retrouve dans l'embryon; le second est retenu au niveau du revêtement ectoblastique placentaire d'où il lui est arrivé exclusivement à travers la paroi des capillaires maternels. Dans ce transfert, les glandes utérines ne jouent aucun rôle, et se montrent totalement imperméables. On obtient les mêmes résultats par injection de trypanblau: ce colorant s'accumule dans l'ectoblaste chorial: on n'en trouve pas trace dans l'embryon lui-même. Ces expériences montrent donc qu'un même organe placentaire jouit d'une perméabilité sélective; il laisse passer les unes, que l'on retrouve dans l'embryon, tandis qu'il arrête les autres en les fixant.

Quant à la zone paraplacentaire, les substances injectées à la mère la laissent absolument intacte: vraisemblablement l'épithélium utérin joue-t-il ici aussi le rôle de barrière imperméable.

Dans le développement des placentas hémochoriaux, nous devons considérer deux phases successives: pendant la première, la prolifération ectoblastique n'est

pas encore irriguée, et absorbe les produits provenant de la lyse des tissus maternels : ainsi voyons-nous de nombreuses hématies maternelles phagocytées et digérées par elle (origine du fer livré à l'embryon). Pendant la seconde, les vaisseaux foetaux gagnent, en rampant le long du pédicule allantoïdien, la prolifération ectoblastique placentaire et s'y ramifient, se mettant en rapports, à travers leur endothélium et la lame de syncytium ectoblastique, avec le sang maternel.

Ici, l'hypertrophie de la zone placentaire de l'ectoblaste chorial ainsi que l'importance de la destruction du tissu maternel à ce niveau avaient surtout frappé les observateurs ; à tel point qu'ils rejetaient toute participation active de la muqueuse utérine dans la nutrition fœtale, celle-ci se laissant simplement digérer ; la notion de placenta maternel, opposée à celle de placenta fœtal, si évidente dans les deux classes précédentes devait, d'après eux, disparaître pour les placentas hémochoriaux. De même, le rôle de la région paraplatcentaire était considéré comme négligeable.

Les recherches entreprises (cf. Jenkinson⁽¹⁵⁾) montrent, au contraire, que chez les espèces à placenta hémochoréal, le tissu maternel intervient activement dans la nutrition de l'embryon, en formant des produits spéciaux et souvent caractéristiques, et que, de ce fait, la notion de placenta maternel doit être maintenue chez tous les Mammifères. Certes, ce placenta maternel ne se présente pas avec le même développement dans toutes les espèces ; même chez les Primates où il est le moins développé et où l'œuf, dès son effraction de la muqueuse utérine, se trouve d'emblée baigné largement dans le sang maternel, il persiste jusqu'à la fin de la gestation, sous forme de cellules déciduales immobiles. C'est chez les petits mammifères à placenta hémochoréal—Rongeurs et Insectivores—que l'on peut suivre le mieux son évolution et étudier ses variations suivant les espèces. Chacun connaît les cellules déciduales de la muqueuse utérine humaine, provenant de la transformation des cellules conjonctives de la couche sous épithéliale de l'utérus. Ces cellules, destinées à être absorbées par l'embryon, se sont transformées en véritables réservoirs de glycogène (cf. Ritter⁽¹⁷⁾).

Bien loin d'être confinées à l'espèce humaine, ces cellules, ou mieux leurs homologues, se retrouvent chez les petits mammifères cités, où leur importance se manifeste à l'évidence. Suivons, par exemple, chez la souris le mode de formation et le comportement de ce que nous appellerons la glande déciduale. (Voir Fig. 1.)

Les premières cellules déciduales apparaissent vers le 8^e jour de la gestation, dans la caduque basilaire, en bordure de la zone d'attaque du cône ectoplacentaire, sous forme de cellules arrondies qui se sont libérées du mésenchyme de cette région. A peine individualisées, elles se chargent de deux sortes d'enclaves : les unes, protidiques, se présentent sous forme de granulations ; les autres, sous forme de flaques, sont constituées par du glycogène. Puis, ainsi transformées, elles se mobilisent, et traversent la paroi des artères maternelles qui vont épancher leur sang dans les mailles de l'ectoblaste chorial transformé en syncytium : elles sont donc entraînées, avec le sang maternel, jusqu'au syncytium placentaire, au contact duquel elles éclatent, mettant en liberté leurs réserves qui sont alors absorbées. La caduque basilaire n'a que peu d'espace pour se développer, et dès le début de la deuxième moitié de la gestation, elle est presque complètement résorbée. Avant qu'elle ne

disparaîsse en entier, et avec elle la glande déciduale à laquelle elle donnait naissance, des modifications surviennent dans le territoire conjonctif utérin localisé entre le muscle utérin et le mésométrium (triangle intermusculaire): c'est par là que pénètrent les vaisseaux utérins, et entr'autres ceux qui se rendent au placenta. Le tissu conjonctif, jusque là banal, de ce territoire limité se transforme et donne naissance à une nouvelle glande déciduale, qui fonctionnera jusqu'à la fin de la gestation: ses

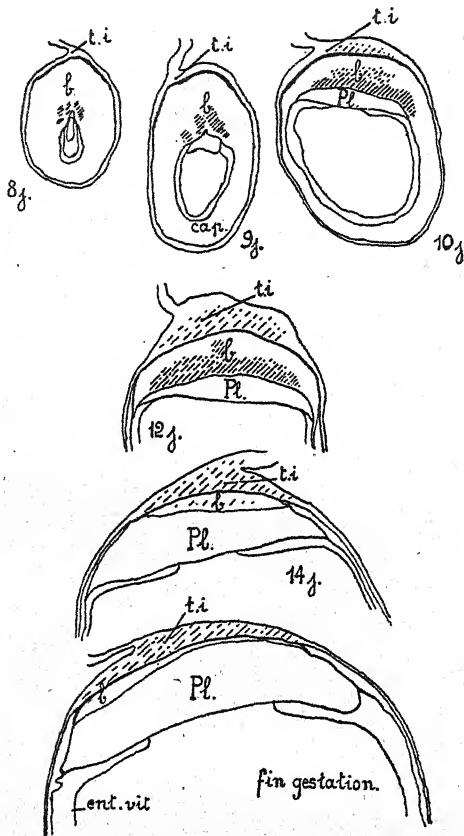


Fig. 1. Coupes transversales schématiques, passant à travers des renflements utérins de souris, d'âge indiqué. Grossissement 6. *b*=déciduale basilaire; *c*=déciduale capsulaire; *ent.vit.*=ento-vitellin; *t.i.*=triangle intermusculaire.

Les parties hachurées sont celles où sont localisées les cellules déciduales.

éléments, bourrés d'enclaves caractéristiques (granulations protidiques et glycogène) traverseront les parois artérielles maternelles pour venir éclater au contact du syncytium placentaire et lui livrer ses produits. Il s'est donc ainsi formé, au cours de la gestation, une glande déciduale vicariante, remplaçant la première épuisée. Un comportement presque identique de la glande déciduale se retrouve chez le rat (cf. (10)); chez un Insectivore, *Nasilio brachyrhynchus*, la glande déciduale vicariante se forme dans le mésométrium (cf. (8)). Chez le lapin, le hérisson, et bien

d'autres mammifères, cette glande déciduale existe bien formée ; elle constitue les formations décrites par les auteurs sous le nom de gânes périvasculaires ou de trophospongia (Hubrecht) : leur caractère sécratoire, leur surcharge en glycogène, permet de faire cette homologie. Ainsi donc s'avère, chez les mammifères à placenta hémochorial, l'existence d'un placenta maternel, opposé au placenta foetal, tous deux intimement soudés l'un à l'autre.

Quant à la partie paraplacentaire de l'ectoblaste chorial, elle peut, suivant les cas, ou bien s'accorder à l'épithélium utérin (Cheiroptères¹, certains Insectivores), ou bien pénétrer dans le conjonctif sous épithelial de la muqueuse utérine en la digérant : nous allons examiner ces deux cas séparément. Mais auparavant, examinons un embryon enveloppé de ses annexes, et fixé dans l'utérus ; les questions suivantes se posent alors d'elles-mêmes à notre esprit : 1°. La partie placentaire assume-t-elle à elle seule la charge de tous les échanges entre la mère et l'embryon, la partie paraplacentaire jouant un simple rôle de membrane de protection ? 2°. S'il n'en est pas ainsi, comment mettre en évidence ce rôle différentiel ? 3°. Dans la partie placentaire elle-même, le syncytium et l'endothélium des capillaires fœtaux sont-ils également perméables ? Les expériences physiologiques entreprises jusqu'ici nous montrent bien que l'on retrouve dans l'embryon certaines substances qui lui ont été cédées par la mère, mais rien ne nous indique l'endroit par lequel ce passage s'est fait. Plus démonstratives sont les expériences pratiquées en injectant à des femelles pleines une solution de bleu de trypan, qui a la propriété de s'accumuler dans certains tissus de façon presque indélébile, et de marquer ainsi la voie qu'il a suivie. Il faut distinguer ici deux grandes classes de placentas hémochoriaux : 1°, ceux où le chorion reste intact, formant une vésicule close, sa zone placentaire pénétrant dans les tissus maternels, sa zone paraplacentaire s'accrochant à la muqueuse utérine intacte : tel est le cas des Cheiroptères et de certains Insectivores : 2°, ceux où le chorion, primitivement clos, a pénétré de toutes parts dans la muqueuse utérine qui l'inclut complètement (tel est le cas de la plupart des Rongeurs et des Primates). Nous y reviendrons plus loin.

Si l'on injecte du bleu trypan à une femelle de Cheiroptère gestante (cf. (12)) on voit le colorant s'accumuler (voir Fig. 2 et Fig. 3) : (a) au niveau du placenta : 1°, dans le plasmoblaste ; 2°, dans les cellules du conjonctif intervilleux (mésoblaste extra embryonnaire) ; (b) au niveau de l'embryon : dans le mésothélium (épithélium exocelomique) entourant, à l'extérieur, l'entoblaste de la vésicule ombilicale dont il est séparé par un feuillet conjonctif fortement vascularisé. Le colorant accumulé dans le plasmoblaste a été extrait du sang maternel ; de même, celui que l'on retrouve dans le mésoblaste intervilleux est la fraction de bleu qui a traversé, sans être fixée, le plasmoblaste et le cytoblaste (ce dernier n'a aucun pouvoir d'accumulation) pour tomber dans les espaces conjonctifs de cette région. Arrivé là, une partie est retenue par les cellules conjonctives, et une autre filtre, à travers le plancher du placenta, dans le liquide exocelomique d'où il est repris et

¹ Nous rangeons ici, en forçant un peu, les Cheiroptères parmi les Mammifères à placenta hémochorial, bien que le sang maternel soit séparé du syncytium placentaire par une lamelle anhydste, reste de l'endothélium des vaisseaux de la mère.

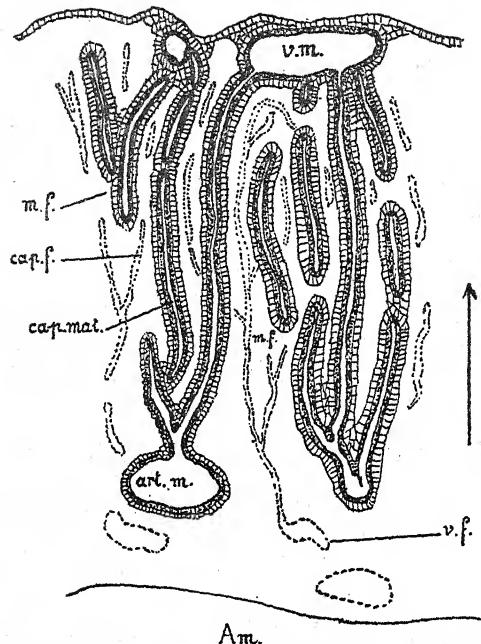


Fig. 2. Coupe schématique représentant un segment placentaire de Chiroptère. *Am.* = cavité amniotique; *Art.m.* = artère maternelle; *cap.f.* = capillaires fœtaux; *cap.mat.* = capillaires maternels dont la paroi, réduite à une lame anhisto, est entourée par les deux couches développées aux dépens de l'ectoblaste chorial: le plasmobilaste (en pointillé) et le cytoblaste; *m.f.* = mésoblaste fœtal; *v.f.* = vaisseau fœtal; *v.m.* = veine maternelle. La flèche indique le sens du courant sanguin maternel.

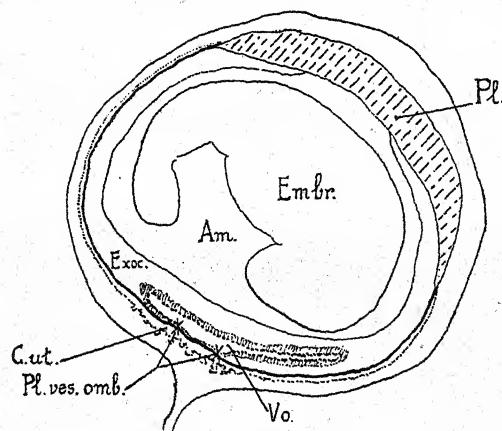


Fig. 3. Coupe schématique passant à travers un renflement de Chiroptère. *Am.* = cavité amniotique; *C.ut.* = cavité utérine; *Embr.* = embryon; *Exoc.* = Exocoelome; *Pl.* = Placenta; *V.o.* = vésicule ombilicale dont la paroi interne, représentée en pointillé, est formée par l'entoblaste vitellin, doublé en dehors d'une couche épaisse mésodermique: les cellules de cette couche qui sont en contact avec l'exocoelome se sont transformées en une couche épithéliale (mesothélium). Le reste (en hachuré) forme en grande partie l'ébauche érythropoïétique. *Pl.ves.omb.* = Plancher de la vésicule ombilicale, formé par l'accrolement direct de l'entoblaste vitellin et de l'ectoblaste chorial: dans le cas où le mésoblaste ne s'infiltre pas entre ces deux couches, le plancher se perfore, mettant en communication la vésicule ombilicale avec la cavité utérine.

fixé par les cellules du mésothélium. C'est bien là la voie prise par le colorant, car : 1°, les cellules endothéliales embryonnaires sont imperméables au colorant : aucune cellule intra embryonnaire n'accumule de colorant ; et pourtant, elles sont capables de fixer le colorant injecté dans l'embryon lui-même ; 2°, le colorant, injecté dans l'exocéloïme, est repris par les cellules du mésothélium et fixé par elles ; 3°, le colorant ne parvient pas à l'exocéloïme par l'ectoblaste de la région paraplacentaire dont les cellules ne contiennent pas de colorant accumulé ; ces cellules sont pourtant capables de fixer du colorant, du moment qu'on l'injecte dans la cavité utérine. Nous pouvons donc conclure de ces expériences que l'ectoblaste est, dans son entier, perméable au bleu de trypan ; mais cette perméabilité n'est pas la seule qui règle au niveau du placenta, le passage de certaines substances de la mère du fœtus ; l'endothélium des capillaires fœtaux joue vraisemblablement un rôle plus important. Au niveau du chorion paraplacentaire, l'épithélium utérin empêche le colorant de parvenir jusqu'à l'embryotrophe. Quant à l'entoblaste vitellin, il semble complètement indifférent, et la vésicule ombilicale qu'il limite se présente comme un organe résiduaire bientôt flétrî qui ne conserve d'importance que par son enveloppe mésoblastique (non compris le mésothélium) où se trouve localisée l'ébauche du système érythropoïétique primordial : rappel des dispositions que l'on trouve chez les Sauropsides. Cette ébauche reçoit sa nutrition, avant que le système circulatoire embryonnaire ne se soit mis en rapports avec le placenta, par l'intermédiaire de l'exocéloïme : le mésothélium puise donc dans le liquide exocélocifique les substances nutritives qu'il cède à l'ébauche érythropoïétique sous jacente.

Toute autre est la disposition chez la plupart des Rongeurs, où une partie de l'ectoblaste chorial, dans sa région obplacentaire, disparaît par fonte cellulaire en un endroit bien localisé, de structure caractéristique, le plancher de la vésicule ombilicale¹. A ce niveau, ectoblaste placentaire et entoblaste vitellin sont intimement accolés l'un à l'autre sans interposition de mésoblaste. Le clivage mésodermique qui donnera naissance à l'exocéloïme épargnera cette région privée de vaisseaux : la fonte cellulaire qui y frappe l'ectoblaste chorial entraîne en même temps celle de l'entoblaste vitellin susjacent : de ce fait, la vésicule ombilicale se perfore d'un orifice plus ou moins étendu qui débouche dans la cavité utérine reformée à ce niveau, par un processus compliqué, sur les détails duquel nous ne nous appesantirons pas ; dans cette cavité s'accumulent les produits sécrétés par l'épithélium utérin ou par les glandes utérines, souvent fort développées en cet endroit. L'orifice de la vésicule peut être étroit et assez tardif, comme chez certains Cheiroptères (cf. (ii)), ou bien large et précoce comme chez certains Rongeurs : dans ce cas, l'embryon s'enfonce dans la vésicule et déprime le feuillet viscéral de celle-ci ; chez certaines espèces (Rat, Cobaye) ce feuillet se referme au-dessus de lui en l'enveloppant en entier, pour se réfléchir ensuite sur la face basale du placenta : en ouvrant alors un renflement utérin par son bord obplacentaire, on tombe immédiatement sur une membrane enveloppant l'embryon, qui n'est autre que le feuillet viscéral entoblastique de la vésicule ombilicale : c'est la

¹ Nous n'envisagerons pas le cas des Primates où le chorion reste intact, et sur lesquels on n'a pas encore poursuivi de recherches histophysiologiques.

disposition dite à feuillets inversés. Cette disposition très spéciale, si variable en étendue, doit être rapportée à une cause unique : la non vascularisation du plancher de la vésicule ombilicale qui, non nourri, disparaît ; cette disparition étant anticipée ou retardée dans l'ontogénèse, suivant les espèces. Ainsi, la vésicule ombilicale se met en rapports secondaires avec la cavité utérine, remplie de produits de sécrétion : cet état rappelle celui où, chez les Sauropsides, elle entoure le vitellus nutritif pour le résorber.

En est-il de même chez les Mammifères à feuillets inversés et particulièrement chez les Rongeurs ? Nous n'examinerons ce point chez ceux-ci que pendant la deuxième moitié de la gestation, où la vésicule ombilicale, largement ouverte, entre en contact avec la cavité utérine. Mais, auparavant, jetons un coup d'œil sur la disposition des annexes fœtales d'un Mammifère à inversion large et précoce des feuillets (voir Fig. 4).

Chez le Rat ou la Souris, que nous choisirons en exemple, le placenta est solidement fixé à la muqueuse utérine dans sa partie mésométriale. Du centre de son plancher part le cordon ombilical qui va se rattacher à l'embryon, entouré de l'amnios ; en dehors de lui règne un espace annulaire étroit (le cœlome externe) limité à l'extérieur par une membrane bien développée plongeant dans la cavité utérine. C'est le feuillet viscéral de la vésicule ombilicale (entoblaste vitellin), doublé intérieurement d'une couche de mésoblaste ; ce feuillet viscéral se replie au niveau de l'embryon pour venir se souder à la partie centrale de la base du placenta qu'il tapisse sur toute sa partie périphérique (entoblaste placentaire) ; entr'eux règne une fente communiquant avec la cavité utérine, le sinus entoblastique¹. Chacune des deux parties de l'entoblaste a une structure qui lui est propre. L'entoblaste vitellin a donc la forme d'une sphère, dont les deux pôles sont diversement différenciés ; le pôle antimésométral lisse et le pôle mésométral portant d'assez longues villosités flottant dans la cavité utérine et le sinus entoblastique : les cellules cubiques ou cylindriques de l'épithélium de l'entoblaste vitellin renferment des granulations plus ou moins nombreuses, de nature protidique. L'entoblaste placentaire se présente au contraire comme un épithélium pavimenteux simple, à éléments très aplatis.

A cette différenciation morphologique correspond, comme nous allons le voir, une différenciation physiologique, sur laquelle les auteurs sont loin de s'accorder.

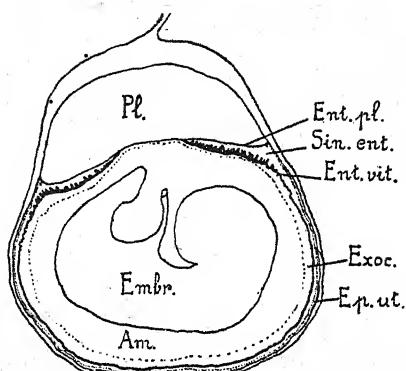


Fig. 4. Coupe schématique passant à travers un renflement utérin de souris, dans la deuxième moitié de la gestation. *Am.* = cavité amniotique; *Embr.* = embryon; *Ent.pl.* = entoblaste placentaire; *Ent.vit.* = entoblaste vitellin; *Ep.ut.* = épithélium utérin; *Exoc.* = exocoelome; *Pl.* = placenta; *Sin.ent.* = sinus entoblastique dans lequel on peut injecter certaines substances pour étudier leur résorption.

¹ Nous passons sous silence une disposition spéciale aux petits Rongeurs : les diverticules de l'entoblaste interombilico-placentaire (Duval) : leur signification est encore trop obscure (cf. (9)).

Alors que pour les uns (Jordan⁽¹⁾) l'entoblaste vitellin serait un organe de sécrétion destiné à former le liquide vitellin, pour d'autres (Asai⁽²⁾) la partie lisse aurait un rôle absorbant, et la partie villeuse un rôle sécrétoire, tandis que pour une troisième opinion (Branca⁽³⁾), la portion villeuse est absorbante. Que, jusqu'au dixième jour de la gestation, l'endoblaste vitellin ait un rôle absorbant, c'est ce que presque tous les auteurs (sauf Jordan) admettent, et c'est ce que confirment les expériences récentes de Brunschwig⁽⁴⁾.

C'est à partir de cette date que l'entoblaste changerait, d'après certains, de polarité pour devenir sécréteur. Les images cytologiques que l'on a invoquées à l'appui de l'une ou de l'autre de ces opinions montrent bien leur fragilité. Pour trancher la question, il faut avoir recours à l'expérience. Les recherches assez anciennes de Goldmann⁽¹³⁾ avaient montré que si l'on injecte du trypanblau à une souris gestante, on retrouve celui-ci accumulé dans l'endoblaste vitellin de l'embryon, alors que l'embryon lui-même n'en contient pas. Une série d'expériences (cf. ⁽⁹⁾) a montré : 1°, le bien fondé des résultats de Goldmann; 2°, que le trypanblau, injecté dans l'embryon lui-même, s'y mobilise pour s'y fixer ensuite, mais jamais dans les cellules de la vésicule ombilicale; 3°, que si l'on injecte ce colorant dans le sinus entoblastique, les cellules de l'entoblaste vitellin l'absorbent et l'accumulent énergiquement. Le bleu injecté à la mère, dans l'expérience de Goldmann, n'est pas arrivé à l'entoblaste vitellin par la circulation fœtale. C'est donc que l'endothélium des capillaires fœtaux du placenta est imperméable au colorant (que l'on trouve localisé dans le syncytium). Il ne reste qu'une seule voie pour l'arrivée du colorant, c'est celle qui emprunte l'épithélium utérin : Eisler⁽⁷⁾ a pu montrer que chez la souris, l'épithélium utérin est perméable à ce colorant; rien d'étonnant à ce que cette perméabilité soit accrue au moment de la gestation et que le bleu passe, sans s'accumuler, à travers les cellules épithéliales de l'utérus pour être déversé dans l'embryotrophe : il est absorbé en même temps que celui-ci par les cellules de l'entoblaste vitellin qui le met en réserve¹.

Il arrive parfois, ainsi que Wislocki^(19, 20) l'a montré dans ses expériences, que le liquide amniotique se teigne en bleu, à la suite de l'injection de bleu trypan à une femelle gestante, et que l'embryon lui-même présente une teinte bleutée, beaucoup plus faible que celle du liquide amniotique : dans ce cas (bien que Wislocki admette un passage du colorant dans le sang de l'embryon) on peut très bien concevoir que la teinte bleue du liquide amniotique résulte du passage, à travers l'entoblaste vitellin, d'une partie du colorant qui n'a pu y être stockée et qui a diffusé dans l'amnios, et que l'embryon s'est coloré secondairement après absorption du liquide amniotique. Tout dernièrement cependant Aron⁽¹⁾ a montré que chez les Rongeurs, le trypanblau se retrouvait dans la circulation embryonnaire, du moment que l'on pratiquait des injections répétées à la mère à la fin de la gestation. Il se produirait donc, à cette période, une augmentation de la perméabilité du placenta; mais ici il faut distinguer entre la perméabilité du syncytium et perméabilité de l'endothélium des capillaires fœtaux du placenta. La première existe dès que le placenta

¹ L'épithélium utérin se comporte donc différemment, envers le trypanblau, chez les Cheiroptères et chez les Rongeurs.

est constitué, et nous avons vu que nous avions toutes raisons de croire à l'imperméabilité de l'endothélium fœtal. C'est à une perméabilisation de ce dernier, à la fin de la gestation, qu'il faudrait donc principalement attribuer les résultats obtenus par Aron.

L'entoblaste placentaire ne paraît à première vue jouer qu'un rôle effacé; cependant, si l'on injecte du trypanblau, ou du citrate de fer ammoniacal dans le sinus entoblastique, on voit ses cellules s'en charger avidement. Bien plus, en injectant au même endroit une suspension de poudre inerte, ou une suspension microbienne (cf. 49) seules les cellules de l'entoblaste placentaire absorbent avidement ces matières étrangères. Il joue donc à la fois un rôle résorbant et un rôle phagocytaire, protecteur: aux différenciations morphologiques de l'entoblaste vitellin correspondent donc des différenciations physiologiques.

Les quelques données ainsi acquises permettent de se rendre compte de la difficulté de l'expérimentation. Elles nous incitent à la prudence lorsque nous voulons étendre à tous les Mammifères des résultats obtenus sur une seule espèce. Elles nous montrent enfin que le placenta, chez certains Mammifères, n'est pas le seul organe d'absorption des produits livrés par la mère.

RÉSUMÉ.

The histophysiology varies in the three different types of placenta, namely the epitheliochorial, the endotheliochorial, and the haemochorionic.

The histophysiology of epitheliochorial placentas is almost unknown. In the case of the other two types, researches have been made which, although fragmentary, allow of the following conclusions being deduced. In endotheliochorial placentas the hypertrophied uterine glands contribute considerably to the nutrition of the embryo. The remainder of the substances, at the expense of which embryonic development is carried out, come from the maternal blood and are absorbed in the region of the placenta by the trophoblastic ectoderm. This absorption is selective: when certain soluble substances are injected into the maternal circulation, some are seen to pass into the embryo, others are stopped by the trophoblast, in which they accumulate.

In the case of haemochorionic placentas the uterine glands supply little material for embryonic development. The embryotrophic substances are derived from two sources. One of these is the maternal cells, which become filled with reserve inclusions (decidual cells) and are absorbed by the trophoblast. A veritable maternal placenta is thus formed, which, in certain species, can change its situation in the course of gestation, being at first localised in the basilar deciduum, then rapidly absorbed, and finally reformed in the region of the mesometrium. The second source is the maternal blood which directly bathes the foetal trophoblast. The latter partially fixes colouring matters injected into the mother, while allowing a certain amount to diffuse into the embryonic mesenchymatous tissue of the placenta. Here the maternal blood comes into contact with the embryonic vessels, the endothelium of which acts as an impassable barrier for the dyes, since these are not found in the

embryo itself, although they pass into the external coelom. In rodents the umbilical vesicle, by means of its vitelline endoderm, acts as an energetic resorber and contributes to the nutrition of the embryo. In the rodents showing inversion of layers the endoderm of the umbilical vesicle is differentiated differently on the placental face and on the inner layer of the umbilical vesicle. Physiological differences correspond to these morphological differences.

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CYANOGENESIS IN PLANTS

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(Received January 8, 1930.)

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INTRODUCTION.

IN a communication made to the *Annales de Chimie* in 1803, Vauquelin recounted that many chemists had recognised an analogy between the odour of prussic acid and that of the flowers and leaves of peaches, and the kernels of related fruits. He related furthermore that Scrader, a pharmacist of Berlin, had that year reported the preparation of prussic acid from bitter almonds and from the leaves of peaches. Vauquelin himself prepared Prussian blue from distillates of bitter almonds and of apricots, and considered that prussic acid was probably present also in plums, cherries and in cherry laurels. A few years later Bergemann (1812) described that, on distillation with water, he had prepared from the bark of *Prunus padus*, an oil resembling that obtained from cherry laurels or bitter almonds, a portion of which had killed a medium-sized dog in ten minutes, but which, on the other hand, when given in small doses, had proved beneficial to certain persons suffering from gout.

The first isolation of a definite cyanophoric principle was made in 1830 by Robiquet and Boutron Charlard, who prepared, from bitter almonds, a crystalline substance which they named amygdalin, from which they obtained prussic acid, a sugar and benzoic acid. They stated definitely, however, that the last-named substance was, in all probability, a result of manipulation, and was not existent as

such in the plant. The chemistry of amygdalin was further studied by Wöhler and Liebig (1837), who described its decomposition by alkalies into amygdalinic acid and ammonia, and showed that, in the presence of a system associated with the plant tissues, which they called emulsin, it could be decomposed into prussic acid, benzaldehyde and "cane sugar." They cited the analogy between the activities of emulsin and the fermentative properties of yeast, and noted the thermolability of the system. Wöhler and Liebig then attempted to obtain emulsin from other plants, including the seeds of the pea and bean but, being unsuccessful, they concluded that such hydrolytic powers were a characteristic property of the protein of the almond seed. Simon (1838), however, claimed that he had obtained a preparation capable of hydrolysing amygdalin from the seeds of sweet almonds, poppies, hemp and black and white mustard. Although the experimental evidence put forward by this author is not now convincing, his supposition as to the wide distribution of emulsin has been fully substantiated.

In 1851 Wicke made a survey of the distribution of cyanophoric substances in a large number of the Rosaceae and, taking the presence of prussic acid as a criterion, concluded that "amygdalin" was almost a universal constituent of the Pomaceae, and was found very frequently in the Amygdaleae. On account of its predominance in young, growing plants, he suggested that prussic acid must be of considerable physiological importance. Many further investigations were carried out by Lehmann (1874), who isolated amygdalin from the seeds of many of the Rosaceae. He was, however, unable to obtain a crystalline product from the vegetative parts of the plants, and concluded that amygdalin as such was absent from these organs, being replaced by an amorphous substance which he called laurocerasin, and which he considered to be composed of one molecule of amygdalin and one molecule of amydalinic acid.

Throughout the nineteenth century, therefore, many examples of cyanogenetic plants were found among the Rosaceae, and prussic acid was accepted as a not unusual constituent of plants of this order. Some opposition was, however, encountered to suggestions that this substance was more widely distributed throughout the plant kingdom. Thus the discovery, made by Jorissen (1883, 1884) of cyanogenetic principles in *Arum maculatum*, in *Glyceria aquatica*, in *Aquilegia vulgaris* and in *Linum usitatissimum* were ignored or discredited, although a cyanide-containing glucoside, namely linamarin, was actually isolated by Jorissen and Hairs (1891).

Such opposition was, however, dispersed by the research carried out at Buitenzorg, at the close of the nineteenth century, by Treub and his colleagues, who identified prussic acid in tropical plants from about twenty natural orders, and furthermore, by the valuable work of Dunstan and Henry (1901, 1902, 1903), who isolated the cyanophoric glucosides lotusin and dhurrin, and made quantitative studies of their distribution. They furthermore rediscovered the discredited linamarin, which they named phaseolunatin.

It is now recognised that prussic acid is present in about fifty natural orders of the higher plants, and ten definite glucosides have been isolated; the cyanophoric

principles of many plants are however as yet unidentified. Among the cryptogams, six ferns have been found to contain glucosides of the amygdalin type (Greshoff, 1909; Mirande, 1918), and prussic acid has been identified in certain basidiomycetes (Offner, 1911; Parisot and Vernier, 1913), while Guyot (1916) has reported the presence of both prussic acid and benzaldehyde in an unidentified phycomycete.

THE BENZALDEHYDE CYANHYDRIN GLUCOSIDES.

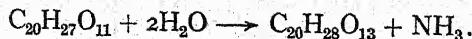
Amygdalin, as stated above, has been shown to be a common constituent of seeds of the Rosaceae, but has not yet been isolated from the vegetative organs of any plant. The chemistry of this substance has been the subject of extensive researches. The non-glucosidal component was shown by Schiff (1870) to consist of benzaldehyde cyanhydrin, but the structure of sugar residue remained unknown for almost a hundred years after the isolation of amygdalin by Robiquet and Boutron Charlard.

E. Fischer (1895) considered amygdalin to be a derivative of maltose, or a "quite similarly constructed biose." He showed that if the glucoside were hydrolysed by a preparation from dried yeast, known to contain maltase, a molecule of glucose was split off, without disturbance of the nitrogenous part of the molecule. The residual substance, which he isolated in a crystalline state, yielded, on hydrolysis with almond emulsin, one molecule of glucose together with one molecule of benzaldehyde and one molecule of hydrogen cyanide, and was therefore a mono-glucoside of mandelonitrile.

Giaja (1910, 1911, 1919) in the course of an investigation of glucosidases prepared from certain invertebrates, showed that if amygdalin were hydrolysed by the digestive juices of the gut of *Helix pomatia*, the biose constituent was split off in an intact state during the first part of the reaction. By stopping the hydrolysis at a suitable stage, he was able to isolate this biose, and showed that it yielded only glucose on further hydrolysis. Since the publication of Fischer's paper (1895) it had been shown that emulsin was without action on maltose; furthermore, the stability of the biose radicle in amygdalin towards acids had been found to be six times as great as that of maltose, a difference presumably too large to be explained by the supposition of an inhibitory influence exerted by the mandelonitrile group (Caldwell and Courtauld, 1907). The biose constituent thus not being maltose, Giaja suggested that it might possibly belong to the trehalose group.

More recently, however, Haworth and Wylam (1923) have shown that the sugar of amygdalin is identical with the glucose-glucoside gentiobiose; moreover, starting from aceto-bromogentiobiose and ethyl *d-l*-mandelate, Campbell and Haworth (1924) have accomplished the synthesis of amygdalin.

If amygdalin be hydrolysed with hot, dilute acids, glucose, benzaldehyde and prussic acid are formed, together with mandelic acid and ammonia. If the hydrolysis be carried out with concentrated mineral acids, the decomposition proceeds as follows:



Amygdalinic acid, $C_{20}H_{28}O_{13}$, on further treatment with hot, dilute, mineral acids, decomposes with the production of *L*-mandelic acid and two molecules of glucose. Amygdalin is thus a compound of gentiobiose and *L*-mandelonitrile.

Dakin (1904), in a continuation of the work of Walker (1903) relative to the action of barya on amygdalin, separated a crystalline racemic isomeride, which he called iso-amygadlin, and which was shown to be hydrolysed by emulsin. Neither this compound, nor the isomeric diglucoside of *d*-mandelonitrile, has as yet been found to occur in nature.

The isolation and identification of the three isomeric monose derivatives of benzaldehyde cyanhydrin was carried out by Bourquelot and his colleagues. Bourquelot and Danjou (1905) obtained from the leaves of *Sambucus nigra* (fam. Caprifoliaceae) a crystalline cyanophoric glucoside which they named sambunigrin, and which was found to be isomeric with the mandelonitrile glucoside prepared from amygdalin by E. Fischer. The same glucoside was discovered almost simultaneously by Guignard (1905), who studied its distribution in the vegetative parts of the plant and the seasonal variation of the concentration at which it is present.

The two optical isomerides of sambunigrin have been found to be apparently restricted to the Rosaceae. Hérissey (1906), in the course of an investigation of the amorphous amygdalin or "laurocerasin" which had been found in the vegetative organs of many plants, was able to isolate from the branches of *Prunus laurocerasus* a crystalline glucoside which he renamed prulaurasin. The physical properties of this substance, namely the melting point and the rotatory power, were shown to be intermediate between those of sambunigrin and those of Fischer's glucoside. Caldwell and Courttauld (1907) prepared prulaurasin from iso-amygadlin by acid hydrolysis, and Hérissey (1907, 2) showed that it could be obtained from iso-amygadlin by the action of a soluble enzyme prepared from yeast. Prulaurasin may therefore be regarded as *d-l*-mandelonitrile monoglucoside.

Fischer's mandelonitrile glucoside was finally isolated by Hérissey (1907, 1) from young branches of *Cerasus Padus* delarb. It was subsequently found by Power and Moore (1909) in the bark of *Prunus serotina*, and by Hérissey (1912) in the branches of *Photinia serrulata*. This glucoside was called prunasin by Armstrong, Armstrong and Horton (1912), and is generally known under this name.

The three isomeric monoglucosides were subjected to a careful study by Bourquelot and Hérissey (1907) who were able definitely to show that sambunigrin is the monoglucoside of *d*-mandelonitrile, prunasin its optical antipode, and prulaurasin the monoglucoside of corresponding *d-l*-racemic compound. Prunasin is therefore the analogue of the widely distributed amygdalin; the diglucosides corresponding to sambunigrin and to prulaurasin, as stated above, are as yet unknown in nature, amygdalin being present in the seeds of plants which contain prulaurasin or prunasin in their vegetative organs.

The synthesis of the monoglucosides of mandelonitrile was accomplished by E. Fischer and Bergmann (1917), who, starting from aceto-bromoglucose and *d-l*-mandelic acid ethyl ester, were able to prepare prulaurasin, and from thence to separate sambunigrin and prunasin by crystallisation.

Another cyanogenetic glucoside containing benzaldehyde and hydrolysable by emulsin was isolated by Bertrand (1906) from the seeds of *Vicia angustifolia*. The constitution of this glucoside, which was named vicianin, was investigated by Bertrand and Weisweiler (1908, 1910) who considered it to be, like amygdalin, a diglucoside of *L*-mandelonitrile. The sugar residue, which these authors called vicianose, was stated to yield one molecule of *d*-glucose and one molecule of *L*-arabinose on hydrolysis with emulsin. The hydrolysis of vicianin as catalysed by the enzyme present in *Vicia angustifolia* and certain other species of *Vicia* is of interest, as, in this process, the intact biose is liberated from the aglucone. The action of the glucosidase resembles therefore that of the digestive enzyme of *Helix pomatia* rather than that of emulsin.

The glucoside vicianin appears so far to be restricted to the genus *Vicia*, and has been identified in four species only. The identity and distribution of a cyanophoric substance in the vegetative organs of these plants has not yet been investigated.

The parahydroxy derivative of mandelonitrile glucoside was isolated from the leaves and stems of the great millet, *Sorghum vulgare*, by Dunstan and Henry (1902) and by them named dhurrin. This glucoside is hydrolysed by emulsin with formation of prussic acid, glucose and para-hydroxybenzaldehyde. On alkaline hydrolysis the decomposition is analogous to that of amygdalin, ammonia and dhurrinic acid being formed. On hydrolysis with hot, dilute acids, dhurrinic acid, $C_{14}H_{18}O_9$, yields glucose and parahydroxymandelic acid.

Since the discovery of prussic acid in *Glyceria aquatica* by Jorissen, many cyanophoric plants have been found among the Graminaceae (Brunnich, 1903; Raybaud, 1913), and it is generally presumed, though with insufficient evidence, that dhurrin is the characteristic glucoside of this order.

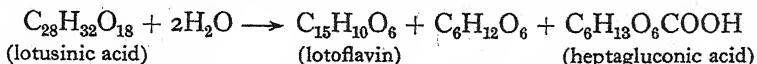
The cyanogenetic glucoside isolated from *Corynocarpus laevigata* (Anacardiaceae) by Easterfield and Aston (1903), and named corynocarpin, was found to yield benzaldehyde on hydrolysis: its complete constitution is as yet unknown. Benzaldehyde and hydrogen cyanide have been found together in plants from about twelve natural orders besides those mentioned above, but lack of definite chemical investigations and isolations as yet precludes the classification of these cyanophoric principles.

LOTUSIN, THE GLUCOSIDE OF *LOTUS ARABICUS*.

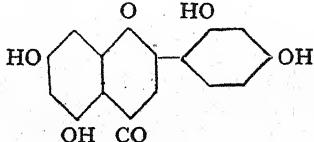
The structure of the remaining known aromatic cyanophoric glucoside is of considerable interest as regards its chemical structure. In all the derivatives of benzaldehyde described above, the cyanide group is combined with the aglucone, forming with it a cyanhydrin. In lotusin, the sugar itself is attached to cyanide, forming a sugar cyanhydrin. The aromatic residue is furthermore of considerable complexity.

Lotusin was isolated by Dunstan and Henry (1901) from the leaves and stems of Egyptian *Lotus arabicus*. In an investigation of the chemistry of the glucoside, the above workers found that on hydrolysis with dilute mineral acids or with the

glucosidase present in *Lotus arabicus*, lotusin decomposes yielding two molecules of glucose, one molecule of prussic acid and one molecule of lotoflavin, a yellow pigment. On alkaline hydrolysis, the glucoside is decomposed into one molecule of ammonia and one molecule of lotusinic acid, the latter, on further hydrolysis with dilute acids, yielding glucose, heptagluconic acid and lotoflavin.



Dunstan and Henry considered that lotusin was probably the ether of lotoflavin and maltose cyanhydrin. They made no definite statement as to the position of attachment of the lotoflavin residue to the sugar-cyanhydrin. The aglucone was shown to be a derivative of phenyl- γ -pyrone, and an isomeride of the flavone derivative luteolin and fisetin, the pigmentary principles of the dyestuffs "weld" and "young fustic" respectively. The constitution of lotoflavin has been represented by the formula



A study of the cyanophoric properties of certain European varieties of *Lotus* was made by Armstrong and his colleagues (1911-12, 1912-13). No isolation of a definite glucoside was reported by these investigators, but it is evident that they considered the principle contained in *L. corniculatus* to belong to the acetone group of cyanogenetic glucosides.

THE GLUCOSIDES OF ACETONECYANHYDRIN.

A cyanogenetic glucoside was isolated from seedlings of *Linum usitatissimum* by Jorissen and Hairs (1891), and by them named linamarin, but the existence of such a substance was disputed by many contemporary workers. In their examination of the chemical properties of the newly isolated glucoside, Jorissen and Hairs showed that, on hydrolysis with boiling mineral acids, prussic acid and a fermentable reducing sugar were produced, together with a volatile substance which gave rise to iodoform when treated with iodine and potash, and which possessed certain ketonic properties.

In 1903 Dunstan and Henry made a systematic study of the cyanophoric principle of a semi-cultivated variety of *Phaseolus lunatus*, grown in Mauritius. In tropical regions the poisonous properties of this plant had been known for many years, and in several medical reports made on fatalities caused by its consumption (Guignard, 1906), prussic acid had been stated to be the toxic principle, Marcadieu, a pharmacist of Réunion, having made this pronouncement as early as 1840. Dunstan and Henry were able to isolate, from the seeds of *Phaseolus lunatus*, a crystalline cyanophoric glucoside, which they named phaseolunatin. This glucoside, on hydrolysis with dilute mineral acids, or with the glucosidase present in the seed, was shown to yield a molecule each of prussic acid, acetone and glucose. On alkaline hydrolysis

it was shown to decompose with the production of one molecule of ammonia and one molecule of "phaseolunatinic acid," $C_6H_{18}O_8$, which, on acid hydrolysis, yields one molecule of glucose and one molecule of hydroxyisobutyric acid. Phaseolunatin has therefore been considered to be a monoglucoside of acetonecyanhydrin.

The hydrolysis of this glucoside is apparently not catalysed by the emulsin of almonds, and Dunstan and Henry for some time considered it to be an α -glucoside. The synthesis carried out by Fischer and Anger (1919) showed definitely, however, that this conception was erroneous.

The identity of phaseolunatin and linamarin was established by Dunstan, Henry and Auld (1906), who furthermore showed that the same substance was present in *Manihot Aipi* and *M. utilissima*. This glucoside, therefore, is distributed throughout several natural orders which are widely separated as regards morphological classification. As yet unidentified cyanophoric acetone-containing glucosides have been found in *Thalictrum aquilegifolium* (Ranunculaceae) and *Nandina domestica* (Berberidaceae).

The plant *Pangium edule* (Bixaceae) has been the subject of much of the important work on cyanogenesis carried out at Buitenzorg. It has been considered by Treub and his colleagues that prussic acid exists partly in the free state in the tissues of this plant, but, while acknowledging that those workers who have had personal experience are most qualified to make authoritative statements, it may be considered, in the light of more modern knowledge of the properties and of the activity of the enzymes present in plants, that the cyanophoric principle in *Pangium* may exist entirely in glucosidal combination.

A glucoside was isolated from *Pangium edule* by de Jong (1909), and was by him considered to be identical with gynocardin, isolated by Power and Gornall (1904) from the seeds of *Gynocardia odorata*. The constitution of this glucoside has not as yet been fully elucidated, but the sugar residue has been shown to be glucose, while the aglucone apparently contains a diketone group (de Jong 1910). A similar glucoside, as yet unidentified, has been demonstrated in several genera of the Passifloraceae.

A comprehensive histological study of the distribution of prussic acid throughout the tissues of *Pangium edule* was carried out by Treub (1896), who furthermore studied the effects of external conditions and variations in the physiological state of the plant on cyanogenesis. The conclusions drawn by this worker as to the biochemical significance of prussic acid will be discussed below.

THE HYDROLYTIC ENZYMES ASSOCIATED WITH CYANOGENETIC GLUCOSIDES.

With rare exceptions, cyanogenetic glucosides are accompanied in the plant by enzymes which catalyse the hydrolysis of the glucoside with liberation of prussic acid and sugar. The action of such enzymes is considered to be responsible for the evolution of prussic acid which takes place on injury of the plant tissue, and for the breakdown of the glucoside in the plant before its components are utilised for metabolic purposes.

The emulsin of almonds, first investigated by Wöhler and Liebig (1837), is generally considered to be a system composed of at least three enzymes. If the course of the hydrolysis of amygdalin by emulsin be followed quantitatively, it is found that the amount of reducing sugar, present at any time in the early stages of the reaction, be compared with the amount of liberated cyanide, the quantity of monose is greater than that which would be produced by a simultaneous liberation of benzaldehyde, prussic acid and glucose (Armstrong, Armstrong and Horton, 1908). It has therefore been suggested that the earliest stages of the action of emulsin on amygdalin are due to a catalyst which brings about the liberation of one molecule of glucose and one molecule of prunasin; this catalyst has been called amygdalase. The second stage of the action consists of the hydrolysis of prunasin, with production of a further molecule of glucose, by an ordinary β -glucosidase, while the decomposition of *L*-mandelonitrile has been attributed by Rosenthaler (1913) to the agencies of a third catalyst, *d*-oxynitrilase. It has furthermore been assumed that the system may include an enzyme amygdalinase, which catalyses the decomposition of amygdalin with liberation of gentiobiose and *L*-mandelonitrile.

Several authors, in particular Armstrong and his colleagues, have considered that the enzymes which accompany the cyanogenic glucosides in the plant are of a specific nature. Thus prunasin and prulaurasin have been said to be associated with "prunase" which presumably would be similar to the second component of the emulsin system, together with the *d*-oxynitrilase. Similarly enzyme in flax has been called linase, and that in lotus, lotase.

In practice, it has been found that emulsin from almonds will bring about the liberation of hydrogen cyanide, from sambunigrin, dhurrin and vicianin as well as from amygdalin, prunasin and prulaurasin. The action of emulsin on linamarin (phaseolunatin) is said to be almost negligible; linase, on the other hand, will catalyse the liberation of prussic acid from amygdalin. Furthermore, as first stated by Simon (1838), enzymes which will bring about the breakdown of amygdalin are present in many plants which are not known themselves to possess any cyanophoric properties. Such enzymes have moreover been shown to be present in certain basidiomycetes which are parasitic on trees (Bourquelot, 1893), in moulds (Gérard, 1893; Hérissey, 1896) and in lichens (Hérissey, 1898).

Many of the views hitherto expressed on specific glucosidases, however, require revision in accordance with the results obtained by Willstätter and his pupils (1923), who have brought forward striking evidence for the opinion that one and the same active group can catalyse the hydrolysis of the aliphatic and aromatic derivatives of β -glucose, the different colloidal carriers with which such an active group is associated in different plants being of importance for its reactivity with the various substrata. In the light of Willstätter's work, the results obtained by Hérissey (1896), in a comparative study of the glucosidases of almonds and that of *Aspergillus niger*, acquire fresh significance.

With the exception of almond emulsin, however, Willstätter and his co-workers made no special study of glucosidases associated with cyanophoric compounds, and their statements are not directed towards such systems in particular. In certain

substances, the cyanogen radicle may introduce a complicating factor, especially when present in combination as a sugar cyanhydrin, as in lotusin. The case of vicianase, which liberates an intact biose from its accompanying glucoside, and the apparently reciprocal action of emulsin and linase, retain a particular interest.

The plant *Sambucus nigra* has been stated to contain an emulsin-like enzyme (Guignard, 1905). Other workers (Bourquelot and Danjou, 1905; Robinson, 1929) have been unable to confirm this statement. If a β -glucosidase should be present in the tissues of this plant, it must be notably less active towards sambunigrin than are similar enzymes from other sources.

THE DISTRIBUTION AND SEASONAL VARIATIONS OF CYANOGENETIC GLUCOSIDES IN PLANTS.

Qualitative estimates of seasonal variation in the concentration of prussic acid were made by Wicke (1851) in a study of the distribution of this substance in the different organs of various Pomaceae and Amygdaleae. Many further data are at present available, but are difficult to summarise.

In the cyanophoric Rosaceae, prussic acid may be regarded as being in general a constant constituent of most of the parts of the plant. The diglucoside amygdalin is present in the seeds, and is said to be replaced in the vegetative organs by the monoglucoside prunasin or its isomer prulaurasin. The young organs, as exemplified by the leaf buds, contain a higher proportion of their total nitrogen as cyanide nitrogen than the adult organs. In common with other nitrogenous substances, cyanide tends to migrate from the leaves before leaf fall. In some cases, a considerable concentration of glucoside is present in the bark, but this rule is not invariable.

Quantitative data as to the variations of prussic acid content in the leaves of *Prunus laurocerasus* have recently been obtained by Godwin and Bishop (1927), who studied the diminution of glucoside in leaves caused to turn yellow in artificial conditions. Further figures as to the seasonal variation of cyanide concentration in this plant, and its diminution during artificial starvation conditions, have been published by Robinson (1929).

In *Sambucus nigra*, no conspicuous seasonal change in the amount of cyanide in the leaf has been observed, and it has furthermore been stated that sambunigrin is present in the withered leaves that are shed at the end of the year. A cyanophoric glucoside is said to be present in immature fruits, but to disappear on their ripening. The identity of this glucoside has not yet been investigated.

Dunstan and Henry, in the course of their researches on fodder plants, showed that in the case of both *Sorghum vulgare* and *Lotus arabicus*, the cyanophoric glucoside was present in the young green plants only, gradually disappearing as the plant approached maturity, and being absent from the seed. Further figures for the concentration of cyanide in young sorghum plants at different stages of growth have been given by Robinson (1929). The seasonal variation of glucoside content in these plants is of economic importance, as, when mature, they are valuable foodstuffs but, when young, they are highly toxic.

The histological and microchemical investigations of Treub (1896) showed that

in *Pangium edule*, cyanide is localised in the phloem and in the pericyclic fibres of the stems, petioles, flowers and fruits, whereas in the seed its presence could be demonstrated in the peripheral cells of the endosperm and in the cotyledons. In the lamina of the foliage leaf it was found in all the parenchyma, besides in the phloem; the basilar hair cells and those containing calcium oxalate being apparently places where cyanide was both formed and deposited. In the bark and pith, the presence of cyanide was generally an accompaniment of morbid phenomena, though "special cells," rich in protein and containing cyanide were found in the bark and pith of various organs, especially in those undergoing a period of arrested development. The concentration of cyanide present was shown to diminish with age in the leaves, and to have disappeared at leaf fall.

Similar histological investigations of *Phaseolus lunatus* (Treub, 1907) showed that, in the case of this plant, cyanide could be obtained in quantity from the leaves and seeds, but was present in traces only in the petioles and branches. As in the case of *Pangium* and of plants belonging to the Rosaceae, the cyanide content diminishes progressively in the adult leaves and disappears in senescence.

There is some evidence that the amount of cyanide present in certain plants may be subject to considerable alteration on cultivation of the plant. The seed of the sweet almond, which is generally regarded as derived by cultivation from the bitter almond, is known to contain practically no amygdalin, although a mandelonitrile monoglucoside appears in the axial organs in the early stages of germination. Similarly, in the seeds of the cultivated varieties of *Phaseolus lunatus*, the concentration of prussic acid is so small that these seeds form a useful foodstuff, whereas those of the wild form are intensely poisonous. A further case in point is that of *Vicia angustifolia*, from the seeds of which vicianin may readily be isolated, whereas the seeds of the cultivated form, namely, *Vicia sativa*, can rarely be induced to yield a colour reaction for prussic acid.

Changes of climate have furthermore been stated to cause variations in the cyanide content of plants. Dunstan and Henry reported that an increase in the concentration of dhurrin was found in *Sorghum vulgare* during spells of drought. A related phenomenon is the striking increase of certain cyanophoric glucosides during frosts, observed by Willaman (1917) for sorghum and by Robinson (1929) for *Prunus laurocerasus*. Armstrong and Eyre (1912) have moreover noted considerable variations in the content of linamarin in flax plants grown in different parts of Europe. Many accounts have been given as to an increase of cyanide in plants after heavy nitrate manuring of the soil, but most of the data available have not been correlated with the ratio of cyanide and total nitrogen, and their value is thus difficult to assess.

THE PHYSIOLOGICAL SIGNIFICANCE OF CYANOPHORIC COMPOUNDS.

Throughout the literature on cyanogenesis, three main ideas may be traced as to the physiological significance of cyanide in the plant. Firstly, in common with other glucosides, the substances described above have been regarded as "excretory products"; secondly, the toxic properties of prussic acid have led many workers

to consider that it serves as a protective substance against animals; thirdly, prussic acid has been regarded as an important and even an obligatory stage in the synthesis of organic compounds of nitrogen by the plant, and thus as a precursor of protein.

In a general discussion as to the function of prussic acid, arguments based on the available data as to the concentration, distribution, and seasonal variation of cyanide throughout the cyanophoric genera lead to widely divergent hypotheses. Throughout such considerations, the perennial difficulty of the plant biochemist is encountered, namely, as to whether the accumulation of any substance in quantity in the plant should be regarded as an indication that it is an important stage in the metabolic process, or whether such a substance is rather a by-product of metabolism, which the organism is slow to utilise.

CYANOGENETIC GLUCOSIDES AS EXCRETORY SUBSTANCES.

The conception that cyanogenetic glucosides are waste products has been developed to some extent by Rosenthaler (1923); the exact opinion of this author is, however, difficult to assess. The same thesis has been expounded by Goris (1921), who has drawn attention to the fact that high concentrations of glucosides are often found in the external regions of old stems, and are eliminated by casting of the bark. He moreover cites cases in which a glucoside is absent from the seed, but present in the early stages of germination, when it may be regarded as a by-product of cell activity.

It has furthermore been considered that, like the animal, the plant may have difficulty in bringing about the breakdown of superfluous cyclic compounds, and that such may be stored as benzaldehyde cyanhydrin or pyrone in an innocuous glucosidal form. A similar explanation is sometimes given for the accumulation of tannins. The function of the sugar residue would be regarded, according to this theory, as analogous to that of glycuronic acid in the animal, that is, as a participant in a protective synthesis.

As against this view, the contention that is improbable, on teleological grounds, that a plant would amass and excrete waste products containing nitrogen, is of some importance. With rare exceptions, the greatest economy with this important element is exhibited by the plant, in striking contrast to the prodigality with which it is wasted by the animal. On the other hand, the apparent inactivity of the alkaloids seems to provide an example of the withdrawal of nitrogenous compounds from the metabolic cycle. The work of Sabaltschka and Jungermann (1925) indicates that the nitrogen of the alkaloids of the seeds of *Lupinus* are utilised to a very small extent. Further considerations based on a study of the variation of alkaloid concentration throughout the vegetative period lead moreover to the opinion that such substances are obligatory but unutilisable by-products of cell metabolism. A similar conclusion might be drawn from the results of Bonnet (1929).

Combes (1918) has, however, drawn attention to the fact that the existence of excretory products in the plant is purely hypothetical, and that substances which are regarded as excretions are those whose function is unknown. He has cited the

following arguments against the evidence that glucosides are to be considered as excretory substances.

(1) The formation or augmentation of glucosides during the early phases of germination, as in *Sorghum vulgare*, *Amygdalis communis*, and *Linum usitatissimum*, may be compared with the accumulation of asparagin and cane sugar in such stages of development. Both these substances are generally considered to be of importance in the metabolism of the plant.

(2) The persistence of glucosides in organs which are about to separate from the plant, as in the leaves of *Sambucus nigra* and of *Passiflora caerulea* is a rare phenomenon; furthermore, it cannot be considered that leaf fall corresponds to a definite physiological state of an organ, as the stage of senescence at which it takes place may vary considerably in the same species in different climates, and with different conditions of temperature, lighting and nutrition.

It may be noted that the localisation of glucosides in the bark, though common, is by no means invariable, as shown by Treub for *Pangium edule*. Furthermore, as the volatilisation of free prussic acid from a living, uninjured plant has never been observed, the glucosidal nitrogen of such plants as sorghum, lotus, and flax, which lose their cyanophoric properties on maturity, must be incorporated in the remaining nitrogenous constituents of the plant.

The general distribution of highly active, cyanide liberating enzymes may be adduced as evidence against the proposed excretory nature of cyanogenetic glucosides. The recent work of Godwin and Bishop (1927) indicates moreover that, from a physiological standpoint, such glucosides are to be regarded as "up-grade" products of metabolism, though this view might be countered by the arguments of Goris that, as such, they are only indicative of the high rate of metabolism of the young cells, which entails a greater production of waste products.

CYANOGENETIC GLUCOSIDES AS PROTECTIVE SUBSTANCES.

The generalisation that the rôle of all glucosides is that of protective substances has been condemned by Hérissey (1923), who suggests that nature would scarcely tolerate agencies which had proved to be so inefficient. Treub (1896) has stated, furthermore, that prussic acid in *Pangium edule* exercises a definite attraction for insects that are injurious to the plant.

Greshoff (1906), however, has described an interesting report of the protective participation of prussic acid in the biological processes of *Arum maculatum*, this substance being stated to narcotise and kill any insects which have penetrated into the plant, after they have performed their task of bringing about pollination.

CYANIDE AS PRELIMINARY PRECURSOR OF PROTEIN SYNTHESIS.

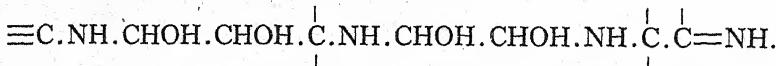
This theory was propounded by Treub (1896) as a result of his extensive microchemical researches as to the distribution of prussic acid in the tissues of *Pangium edule*. The presence of this substance in the phloem was considered to indicate its importance as a translocatory material. It was shown to be absent from the immature phloem elements at the growing point, but to be present in this region in the

pericyclic fibres, which were considered to act as conductors of plastic nitrogenous substances in places where the phloem elements were insufficiently well defined to act in this capacity. Ringing experiments showed furthermore that it was present above the incision, but gradually disappeared below, thus indicating that the site of its formation was the leaf.

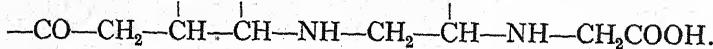
The facts of the occurrence of cyanide in the phloem and pericycle; the absence of protein from young "special cells," the basilar hair cells and the oxalate-containing cells, and the disappearance of cyanide from leaves during senescence or on darkening of the plant, were regarded by Treub as evidence that cyanide is a precursor of protein. He concluded further that the notable accumulation of cyanide in the basilar hair cells and the oxalate cells was an indication that its formation was normally related to photosynthetic processes.

The relationship between the activity of chlorophyll and the production of glucosides has been postulated by many workers who believe that the latter substances are of importance in the active metabolic processes of the plant, and the case of *Aquilegia vulgaris* is often cited as one in which the cyanophoric principle (probably linamarin) is restricted to the organs which contain chlorophyll. On the whole, however, the relationship between glucoside production and photosynthesis seems to be indirect, and it has been stated, in the case of plants which contain prussic acid in the vegetative organs only, that the production of the cyanophoric glucoside takes place in seedlings which have germinated in darkness.

Some theoretical support has been received for the hypothesis of Treub. Gautier (1884) considered that nitric acid, which might be liberated by carbonic acid or by organic acids from nitrates absorbed from the soil, as suggested by Bach (1896), might react with formaldehyde, produced in photosynthesis, with formation of water, carbon dioxide and prussic acid. Condensation with formaldehyde might then occur, resulting in chains of the following type:



In these, $\equiv \text{C} \cdot \text{NH}$ groups might undergo hydrolysis, and $-\text{CHOH}$ and $-\text{NH} \cdot \text{CHOH}$ groups reduction, giving rise to



If ammonium salts, rather than nitrates, provide the original source of nitrogen for the plant, it might presumably be considered that hydrogen cyanide is produced by dehydration of formamide obtained from ammonium formate.

Experiments carried out by Combes (1918) in order to test whether amygdalin could serve as a source of nitrogen for the higher plants led to definitely negative results, the cyanophoric substance being shown to exert a toxic influence. The fact, however, that cyanide disappears very rapidly from the leaves of *Prunus laurocerasus* on "starvation," that is, from cut leaves kept with their petioles in water and in darkness or diffuse light (Godwin and Bishop, 1927; Robinson, 1929), seems to provide evidence for the assumption that, in this case at least, the plant is able

readily to utilise nitrogen in this state of combination; the available data, however, do not clearly indicate into what form such nitrogen is first changed in the course of starvation.

In the case of *Sorghum vulgare*, Willaman (1917) has shown that a great increase in the amount of cyanide present can be brought about by frosting and by anaesthesia. If cyanophoric compounds are to be regarded as "upgrade" rather than as decomposition products, an analogy might be cited between such effects and those obtained by Zaleski (1901), who reported an increased formation of protein in seedlings of lupin after ether anaesthesia.

The disappearance or diminution of cyanide on cultivation of a plant has been considered by some to indicate that this substance cannot be regarded as a fundamentally obligatory constituent of the plant. To those, however, who regard the chemical composition of an organism as intrinsically related to its structure, the views expressed by Dunstan and Henry (1903) are more acceptable, namely, that the improved conditions of nutrition and environment in cultivation cause a stimulus to metabolism, leading to a more rapid utilisation of plastic substances, so that their accumulation in the plant tends to become less obvious.

Owing to the predominating number of plants in which prussic acid is indetectable, the majority of opinions expressed have been unfavourable to the view that this substance is an universal preliminary in the synthesis of organic nitrogen compounds in the plant. As one of the very rare constructive suggestions, however, the idea is not unworthy of consideration, and although the postulation of indetectable stages in any process is unsatisfactory, it is inevitable that such assumptions must often be made in our present incomplete state of knowledge.

SUMMARY.

Prussic acid, identified during the early part of the nineteenth century as a constituent of many members of the Rosaceae, is now known to be present in plants from about fifty natural orders. It is generally considered to exist in the living plant exclusively in glucosidal combination, but certain workers, including Treub and his colleagues, have postulated the existence of free cyanide in plant tissues.

Ten cyanophoric glucosides have been isolated in crystalline form. Of these, seven are derivatives of benzaldehyde cyanhydrin; linamarin and gynocardin contain ketone groupings, and lotusin, the glucoside of *Lotus arabicus* is a derivative of γ -pyrone combined with a sugar cyanhydrin. The glucosides prunasin, sambunigrin, prulaurasin, linamarin and amygdalin have been prepared synthetically.

Amygdalin, prunasin and prulaurasin seem to be restricted to the order Rosaceae, sambunigrin to the Caprifoliaceae, and dhurrin to the Graminaceae; while vicianin has so far been found only in a few species of the genus *Vicia*. Linamarin, on the other hand, has been found in several natural orders, widely separated in morphological classification.

In the plant, cyanophoric glucosides are, with few exceptions, accompanied by active, cyanide-liberating, hydrolytic enzymes. The conception as to the specificity

of such enzymes, as postulated by many authors, may need some modification in the light of the recent work of Willstätter and his pupils.

The concentration and seasonal variation of prussic acid in the plant show considerable differences in the several cases known. In general, the concentration is greatest in young, growing organs; *Sambucus nigra*, on the other hand, shows little seasonal variation in cyanide content. In other cases, cyanide has been shown to exist in quantity in such tissues as the bark. In certain of the Rosaceae, cyanophoric glucosides appear to be constant constituents of the plant throughout its life cycle, while in the Graminaceae and in *Lotus arabicus*, they disappear at maturity and are absent in the seed.

In some cases, the concentration of prussic acid in the plant has been shown to be diminished by cultivation (*Amygdalis communis*, *Vicia angustifolia*, *Phaseolus lunatus*). Climatic conditions, especially drought, have furthermore been stated to cause variations in the content of dhurrin in *Sorghum vulgare* and of the cyanophoric glucoside of *Lotus corniculatus*. Temperatures below freezing point have been observed to cause a rapid and conspicuous increase in the glucoside content of *Sorghum vulgare* and in *Prunus laurocerasus*.

The function of prussic acid in the plant is uncertain. Since the researches of Treub on the localisation of cyanophoric substances on *Pangium edule*, many workers have considered that cyanides may represent the first stage in the synthesis of organic nitrogen compounds by the plant. The rapid disappearance of cyanide under conditions of starvation, as observed in *Prunus laurocerasus*, supports the view that such nitrogen is readily utilizable by the plant. Other workers regard cyanophoric glucosides as excretory products, or, in virtue of their poisonous properties, as protective agencies.

Quantitative investigations as to nitrogen partition in cyanophoric plants at various stages of growth might help to elucidate the important question as to the participation of prussic acid in protein synthesis by the plant. At the present time, adequate data on this subject are not available.

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THE BIOCHEMICAL ASPECT OF THE RECAPITULATION THEORY

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(Received January 14, 1930.)

(With One Text-figure.)

THIS subject may perhaps best be approached from the consideration of the transitory functions of embryonic life. If W. Preyer had been asked why, in 1885⁽⁴⁰⁾, he entitled his book *The Special Physiology of the Embryo*, he might well have replied that it was because in point of fact the physiology of the embryo does differ in certain ways from the physiology of the adult of the same species. This is not only because embryonic development involves a series of steps, each one more complicated than the one before, but also because the embryo has certain structures which the adult has not, and the embryo can perform certain functions which the adult cannot. Wintrebert⁽⁵⁴⁾ who has much extended our knowledge of the neurology of foetal life, came across several such functions in his own field and summarised them as follows: (1) the aneural rhythmic contractions of selachian myotomes, (2) the aneural ectodermal irritability of amphibian embryos, (3) the undulatory movements of selachian embryos (Rohan-Beard cell reflexes), (4) the hatching enzymes of teleostean fishes, insects, amphibia, and cephalopods. These functions, he considered, formed part of a special physiology of the embryo and could not be regarded as in any way a "rappel ancestral." The first three he thought might be of nutritive significance, assisting in some way the nourishment of the embryo. The fourth was obviously involved in the hatching process and in many cases of a remarkably striking transitoriness; a special gland developing on the head of the embryo, pouring forth a protease into the perivitelline cavity and then undergoing atrophy immediately after emergence. Other hatching mechanisms, such as the egg tooth in insects and the explosive bomb of the *Reduvius* bug, come into the same category, but of the more subtle transitory functions perhaps the most interesting is the transitory liver of birds and mammals.

It seems clear that in the early stages of development the embryonic liver is unable to store glycogen as it does in later life, so that some other cells have to do the work—these are outside the embryo altogether, in the yolk sac and blastoderm in reptiles, fishes, and birds, and in the placenta in mammals. In the hen's egg, for instance, at the time of laying there is very little glycogen (Sakuragi⁽⁴⁶⁾, and others),

but it is synthesised regularly all through development. Yet for the first two-thirds of the time, by far the greater part of it is not in the embryo at all, and it is not until the end that the embryo begins to store it. This is the phenomenon of the *foie transitoire* first discovered by Claude Bernard⁽⁵⁾. Here again, there is no need to suppose that anything more than a kind of scaffolding is concerned—the egg first of all runs up a series of poles and boards, some of which take necessary strains while the embryo is being built, and are afterwards removed. There can be no doubt that the yolk sac has important metabolic functions to perform in the hen's egg, for xanthine oxidase (Morgan⁽³³⁾) and ovomucoidase (Needham⁽³⁵⁾) are to be found in it long before they make their appearance in the embryo itself. The property of swimming in a watery amnion and of respiring through the allantoic vessels, in the case of terrestrial embryos, would presumably come under the head of transitory functions, and the membranes which are left behind in the egg when the chick "passes forth to enjoy the outward aire" are certainly analogous to the poles and ropes lying on the ground beside a finished house. Another instance of the scaffolding type of mechanism might perhaps be drawn from Shikinami's discovery⁽⁴⁸⁾ of insulin in the yolk of the avian egg, with its later confirmation by Hanan and Pucher⁽²²⁾. At the time of laying there is none in the embryonic cells, and the islet tissue, of course, does not develop until much later, but the yolk especially has a complicated series of evolutions to perform with respect to carbohydrates during the early days of development, and a regulatory hormone which the embryo cannot supply is apparently supplied for it by the parent organism. Again, there is the possibility that the unsaturated fatty acids of the yolk are used in the early stages in preference to the saturated ones (Needham⁽³⁶⁾), into which the embryo is probably incapable of introducing double bonds before the fifteenth day. Indeed, almost the whole of the chick's embryonic nutrition could be looked at from this point of view conformably with the observation of William Harvey⁽²³⁾: "For while the foetus is yet feeble, Nature hath provided it milder Diet, and solider Meats for its stronger Capacity, and when it is now hearty enough and can away with courser cates, it is served with Commons answerable to it."

But sometimes in the survey of transitory functions one comes across instances which do not seem to have anything to do with scaffolding processes. One of the most striking of these is the way in which the chick embryo excretes the major part of its nitrogen, first of all as ammonia, then as urea, then as uric acid (Needham⁽³⁷⁾, Fiske and Boyden⁽¹⁴⁾, Targonski⁽⁵¹⁾). The biochemist cannot fail to be impressed by the corresponding phylogenetic fact that invertebrates excrete for the most part ammonia, and fishes urea, and in this way comes up against the theory of recapitulation almost without knowing it. When he makes some examination of that theory he finds that there is a good deal to be said about it from the biochemical point of view.

As we now know it, it originated in the early years of the nineteenth century with Meckel, Serres, the elder Agassiz and von Baer. In those days the statement made was simply that the embryos of all species were more alike than the adults, and that the younger you took the embryo the more alike they were, so that after

the advent of the cell theory it was affirmed that when you got back to the zygote, the egg cells of dog and duck were indistinguishable morphologically. This was really not a very wide departure from Aristotle's generalisation⁽²⁾ that in ontogeny the more general characters as a rule appear before the more specific ones. The following passage from von Baer⁽⁴⁾, already well known, became still more famous by being quoted by Darwin in the *Origin of Species*⁽¹¹⁾: "The embryos of mammals, of birds, lizards, and snakes, and probably also of chelonia, are in their earliest states exceedingly like one another, both as a whole and in the mode of development of their parts: so much so, in fact, that we can often only distinguish the embryos by their size. In my possession are two little embryos in spirit, whose names I have omitted to attach, and at present I am quite unable to say to what class they belong. They may be lizards or small birds, or very young mammals, so complete is the similarity in the mode of formation of the head and trunk in these animals. The extremities of these embryos are, however, absent still. But even if they had existed in the earliest stage of their development we should learn nothing, for the feet of lizards and mammals, the wings and feet of birds, no less than the hands and feet of man, all arise from the same fundamental form." The statement of the case was fully accepted by Darwin himself and his contemporary exponents of evolution. "Community in embryonic structure," he said⁽¹²⁾, "reveals community in descent." This was unaffected by cases where the relation did not hold, dissimilarity of development did not mean discommunity of descent, because developmental stages might be missed out or so modified by the requirements of embryonic life as not to be recognisable. This process was called "caenogenetic modification" by Haeckel⁽²¹⁾.

The attraction of applying evolutionary succession to embryonic life or rather of superimposing phylogeny *en bloc* on to ontogeny was too great to be resisted, and the recapitulation phenomena were taken to mean that more ancient adult types were to be found thinly disguised in the ontogenies of more recent types. Thus Darwin himself said⁽¹³⁾: "As the embryo often shows us more or less plainly the structure of the less modified and ancient progenitor of the group, we can see why extinct and ancient forms so often resemble in their adult state the embryos of existing species of the same class." And Haeckel⁽²¹⁾ went further still, making the assumption that the embryo was, in Garstang's phrase⁽¹⁸⁾ "nothing but an animated cinema-show of ancestral portraits." The embryo was regarded as a picture, more or less obscured, of the progenitor of its class.

In later times, the theory of recapitulation in this form came in for a great deal of destructive criticism (for a review of which see Russell⁽⁴⁵⁾). Adam Sedgwick⁽⁴⁷⁾ attacked the view which emphasised so much the similarity between embryos of different animals. Taking the case of the fowl and the dogfish he showed that the differences were at least as striking as the resemblances; the blue yolk of the one, the yellow yolk of the other; the embryonic rim and blastopore of the fish, the absence of these in the chick; the six large gill slits bearing gills on the one hand, the four rudimentary clefts on the other; the small head, straight body, and long tail, as opposed to the enormous head, cerebral curvature, short tail, and so on. Sedgwick

pointed out that characters of embryos had varying importance because of their varying value in classification, and proposed that von Baer's law should be modified so as to read: "Embryos of different members of the same group often resemble one another in points in which the adults differ, and differ from one another in points in which the adults resemble, and it is difficult if not impossible, to say whether the differences or the resemblances have the greater zoological value." And Sedgwick was able easily to show that not only in two embryos so far apart as duck and dogfish but also in embryos as nearly related as duck and chick, it was really not very hard to distinguish between them, even in the early stages.

But in spite of all the criticism which the theory of recapitulation had to undergo, it did not fail to survive, for as Sedgwick admitted, there *are* resemblances between embryos, and structures reminiscent of more ancient or less organised types *do* exist in embryonic development. The chick does at a certain stage possess pharyngeal clefts, a tubular piscine heart, a piscine arrangement of the cardiac arterial system, a cartilaginous endo-skeleton, oro-nasal grooves, a notochord, and, as Goldby (20) has recently found, a set of unilateral ectodermal placodes corresponding to the acoustico-lateral system of the anamniota. The "flat" pleuronectid does, as an embryo, have a shape like any other fish, and the sole about to leave its egg has two eyes placed symmetrically one on each side of its head. The embryo *Icthyophis* does have legs like any other amphibian. The crab at the megalopa stage does have an abdomen as large as a lobster or a prawn, the limpet embryo does have a spiral typically gastropod shell, which is lost on the formation of the conical shell of the adult. The whalebone whale, in its embryonic form, does have teeth, which it afterwards loses, and so have those plates, equivalent to the placoid scales of fishes, which, though at first separate, afterwards fuse to form the amphibian vomer. There is no need to extend further the list of examples; it suffices to show that there are recapitulation phenomena and that they need an explanation.

The explanation given by the early evolutionists was, of course, purely in terms of heredity. A given animal stamped its form upon its descendants in the act of generating the first of them, and although they might succeed in greatly modifying it, traces of it would always remain to arouse the interest of the embryologist. This pre-occupation with heredity accounts for the hostility with which Wilhelm His and his associates were regarded when they began to bring causal concepts into embryology and to work out the mechanics of development. Embryologists at that time did not want to know how the invagination of a gastrula could be brought about in terms of physical and chemical forces; they wanted to go on hunting for ancestral portraits in the miscellaneous collection of pictures presented by the developing embryo. D'Arcy Thompson has described the incredulity and opposition with which the views of His were met (24) in the 'eighties. As Garbovski put it (17): "it is absurd to treat the living being as if it were made up of vesicles, cylinders, and plates, and not of vital units." Embryologists such as Hertwig and Balfour held that a sufficient causal explanation of one developmental stage had been given when the immediately preceding stage had been adequately described. "My own attempts," wrote His, in 1888, "to introduce some elementary mechanical or

physiological conceptions into embryology have not been generally agreed to by morphologists. To one it seemed ridiculous to speak of the elasticity of the germinal layers; another thought that by such considerations we put the cart before the horse; and a more recent author states that we have better things to do in embryology than to discuss tensions of germinal layers and similar questions, since all embryological explanations must necessarily be of a phylogenetic nature. This opposition to the application of the fundamental principles of science to embryological questions would hardly be intelligible if it had not a dogmatic background. No other explanation of living forms is allowed than heredity and any which is founded on another must be rejected—yet to think that heredity will build organic beings without mechanical means is a piece of unscientific mysticism." Experimental embryology showed that it was capable of looking after itself, but the recapitulation theory continued to captivate the imagination of morphologists and if mere heredity, without any other mechanism, was inadequate to explain it, other and better explanations had to be searched for. This revolt against the merely hereditary form of the recapitulation theory has taken several aspects.

As Sedgwick pointed out, the process of embryonic development resembled not so much a few new modifications embedded in a host of ancestral stages, but a few ancestral traces embedded in a host of new modifications. In ontogeny, as it were, ontogeny rather than phylogeny was supreme, whereas the simple recapitulation theory assumed that the repetition of ancestral characteristics in embryogeny was the intelligible rule and that their omission was the exception which required explaining whenever it occurred. "The balance of evidence appears to me," Sedgwick said (47), "to point most clearly to the fact that the tendency in embryonic development is towards directness and abbreviation and to the omission of ancestral stages of structure, and that variations do not merely affect the last period of life when they are of immediate functional importance to the animal, but on the contrary that they are inherent in the germ and affect more or less profoundly the whole of development." From the point of view of the ancestor, as it were, the embryo of a later species has got much more out of hand than the older workers would have liked to think, and is going ahead on its own with an insufficient respect for the ancient form on which it was supposed to be modelling itself. Considerations of this kind led Garstang (18) and, later, v. Franz (16) to reverse completely the recapitulation theory, and to say that instead of phylogeny creating ontogeny, ontogeny creates phylogeny. By the time that Garstang was writing, in 1922, practically every biologist, except MacBride and a number of palaeontologists, had given up the notion, so dear to Haeckel and his associates, that an embryo recapitulates adult stages in its development, and regarded the stages recapitulated as embryonic or larval. As Garstang said, Haeckel only paid attention to the phylogenetic succession of adults, and forgot that other phylogenetic succession, the list of zygotes or fertilised eggs. Fig. 1, modified from Garstang's paper, shows the two sequences, A_1 to A_9 being the adult forms from worm to mammal, Z_1 to Z_9 being the egg cells in each case. "Through the whole course of evolution every adult metazoan has been the climax of a separate ontogeny or life cycle which has always intervened between

adult and adult in that succession of forms which Haeckel termed phylogeny. The real phylogeny of metazoa has never been a direct succession of adult forms but a succession of ontogenies or life cycles." Thus in Fig. 1 the coelenterates, passing in their ontogeny from, say, Z_2 to A_2 produced at a certain point eggs, nearly all of which returned to the starting-point Z_2 and repeated the whole process, but one of which took a startlingly different course, to Z_3 , a course which led eventually after a new ontogeny, to a new type of adult, a coelomate, A_3 . In each case the organism moves from its Z to its A along a course, the same and yet not the same as the course of its predecessor. Phylogeny, in Haeckel's sense, is

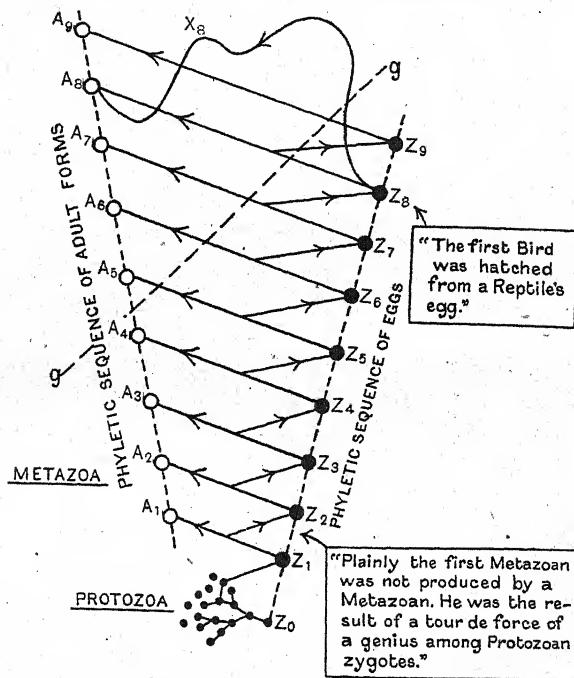


Fig. 1.

the result, the record, the effect, of the ontogenies which precede it all the time, so that ontogeny is not the recapitulation of phylogeny but the recapitulation of previous ontogenies in so far as it does not diverge from them on lines of its own. Phylogeny is anything but the "mechanical cause", as Haeckel called it, of ontogeny; on the contrary the latter creates the former. "Ontogeny," said Garstang, "proceeds through successive grades of differentiation by which tissues, layers, organs, and parts, together with ordinal, family, and generic characters, are more or less successively established. As differentiation increases, the combination of parts exhibited at successive stages resembles more or less distinctively the combinations characteristic of successive grades of evolution represented in our schemes of phyletic classification," represented in Fig. 1 by the parallel ontogenies at ever-increasing heights. "To that limited extent the ontogeny of a given animal is the

recapitulation of its phylogeny and may be said in the true sense of the word to recapitulate phylogeny, *i.e.* to sum it up, to recall the main phases of it. This is the parallelism observed by Meckel, von Baer, and others, and expressed in evolutionary terms. It exists and is undeniable."

In other words ontogeny reproduces successive grades of ancestral differentiation, not because adult types have been included in it, but because each ontogeny is a modification, within limits, of its predecessor; and it was just by those predecessors that the phyletic chain of adults was organised and equipped. The chick embryo does not, then, pass through a fish stage, but through a stage resembling the embryonic form of a fish, and it is consequently at the cost of biological accuracy that writers such as Aldous Huxley, wishing to emphasise the contrast between man's activities and his origins, refer to him effectively enough as an "ex-fish." But what is it that governs the degree of parallelness between the successive ontogenies of fish, reptile, and mammal; why should the chick embryo copy the piscine gill clefts, and not its blastodermal arrangements or its long tail? Why are some structures and some functions left out and others kept in? Why should the chick retain gill slits at a certain stage and yet the snake not retain fore limbs at any stage? Why should not an ontogeny move off in a totally different direction, as in the line $Z_8-X_8-A_8$ of Fig. 1, retaining no reminiscences of previous ontogenies at all and using nothing but new processes previously unknown? Garstang, busily engaged in destroying the last vestiges of the ancestral portrait gallery, did not give any particular answer to this question, although from the causal or physico-chemical point of view it would be difficult to ask a more fundamental one.

Probably the answer is that an organism only recapitulates as much of previous ontogenies as it has to. The parallelism seems to be capable of a purely mechanical explanation, an explanation which more or less abolishes the mysterious action of ancestral heredity, the dead hand of tradition, a concept hard indeed to express in satisfactory causal terms. It looks as if organs are only recapitulated in so far as they are necessary for the development of the ones which are required in the ontogeny in question, and all the other old ones disappear. The first suggestion that this was so is apparently due to Kleinenberg⁽²⁶⁾ who in 1886, in his work on the embryology of annelids, introduced what he called a "substitution theory," a proposal that new organs or structures arise in an ontogeny, not by a morphological variation of the replaced or preceding organ but having a genetic relation to it such that the new can only arise in an organism containing the old, *i.e.* that it is dependent on the prior existence of the replaced organ. This idea was taken up and given prominence by Milnes-Marshall in 1890⁽³²⁾ who instanced the notochord as an example of Kleinenberg's view. It is formed by change of function from the wall of the digestive canal, but while it is the sole skeleton of the lowest vertebrates, it is the earliest developmental phase of the skeleton in the higher forms. The notochord gives place directly to no other organ but is gradually replaced by other and unlike structures; this is substitution. The cartilaginous skeleton which replaces it is only intelligible, according to Kleinenberg, through the previous existence of the notochord, while in its turn substitution replaces the bony for the cartilaginous skeleton. A rough

analogy could be taken from the development of man's devices for setting a projectile in rapid motion; the bow and arrow was an improvement on the sling but did not arise by any increase in the perfection of the latter, and similarly the gun was an improvement on the bow and arrow, but yet originated from a quite independent principle, by substitution, as Kleinenberg would have said, and owed nothing to the former save the stimulus which led to its formation.

The nature of this stimulus was not made clear by the earlier embryologists. As Marshall said: "Kleinenberg's theory acquires special interest from the explanation which it offers of recapitulation as a mechanical process, through which alone it is possible for the embryo to attain the adult structure. If it be really true that each historic stage in the evolution of an organ is necessary as a stimulus to the development of the next succeeding stage, then it becomes clear why organisms are constrained to recapitulate." In some presentations, e.g. Sedgwick's, the retention of ancestral characteristics was also regarded as determined by their physiological value, only in a more crude manner than Kleinenberg's. According to Sedgwick the general tendency was for ancestral structures or organs to be blotted out unless they were of some value to the organism in its free-living larval state and were therefore perpetuated. He explained the instances of recapitulation in embryonic life by an absorption of a larval or immature free stage into the prenatal period, such as occurs in tree frogs, so that the valuable ancestral characters having been preserved for a long period in the larva by natural selection were retained in the subsequent embryo simply as structures at one and the same time rudimentary and vestigial. On this view embryonic development, as far as it is a record at all, is a record of structural features of previous larval stages, which in turn would be all that natural selection had left of the original ancestor. "Characters which disappear during free life," said Sedgwick, "disappear also in the embryo, but characters which though lost by the adult are retained in the larva may ultimately be absorbed into an embryonic phase and leave their traces in embryonic development." The weakness of this point of view was that it treated embryonic organs as functionless and Sedgwick thought that as this was the case there could be no reason for the retention of any ancestral conditions. But everything that experimental and physiological embryology has since discovered goes to show that Harvey was right in denying that foetal organs "keep holiday." The tendency would now be to regard the developing embryo as an exceedingly complicated machine, in which there are no idling wheels and layshafts, although no doubt all the mechanisms are not in gear at the same time, and Kleinenberg's outlook is therefore the more acceptable.

The nature of the stimulus involved in the substitution of a new organ for an old one, and present in the recapitulated structure, was for a long time obscure. Garstang interpreted Kleinenberg as meaning nothing more than scaffolding: "Backbone replaces notochord and bone replaces cartilage in present, as, doubtless, in past, ontogeny, for the former organ or tissue is still necessary as scaffolding for the later one, and the constant appearance of gill slits in the ontogeny of terrestrial vertebrates is but another illustration of the same phenomenon." But a better

illumination has come through the work of Spemann and his associates on organiser phenomena, and it is very permissible to suppose, though as yet there is no direct experimental evidence for it, that just as the organiser in the dorsal lip of the amphibian blastopore generates a neural plate and neural folds, so an organiser in the cells of the notochord generates bony spinal structures. The organisers in these ancestral structures would thus be of the third or fourth grade and the structures themselves would only be recapitulated because they contained the essential formative stimulus for the younger structures. "Old adult characters," as Garstang puts it, "are eliminated from ontogeny unless required as temporary bases for the new characters." Thus the reason that the course $Z_8 - A_8$ is taken in Fig. 1 and not $Z_8 - X_8 - A_8$ is that to throw away the recapitulatory similarities would be to abandon the tools which normally aid the work in question.

It is of course important to remember that not all the processes which have in the past been put down as recapitulatory ought really to bear such a designation. Ford and Huxley⁽¹⁵⁾ have shown that differential growth-rates of organs and differential rates of physiological processes such as the deposition of the melanin in the eye of *Gammarus*, will sometimes fully account for phenomena presenting at first sight every appearance of a "rappel ancestral."

Having now taken stock of the theory of recapitulation as it comes to us from the hands of the morphologists, it would appear as if they have brought it just to a position in which it could be incorporated into a chemical embryology. For if ancestral ontogenetic stages are only recapitulated because they are useful, and useful in a perfectly definite, almost endocrine, sense, then the concept of embryonic development is one and the same for the morphologist as for the experimentalist. Future research will have to unravel the details of the action of the formative factors of the recapitulated structures, and it would be unwise to prejudge the issue regarding the physico-chemical nature of organisers in general. But whether they are chemical substances resembling hormones, propagated disturbances not dissimilar from the nervous impulse, gradients of "physiological activity" or something much more subtle than any of these, the parallel between organiser phenomena and substitution effects associated with recapitulated structures is too close to be disregarded and offers, at least, an attractive working hypothesis.

Now there are a number of phenomena of a biochemical order which occur in embryonic development and which have been brought forward from time to time as examples of the operation of the recapitulation law. How do these fit in with the general scheme which has been outlined in the preceding pages? There is first of all the ammonia-urea-uric acid sequence in the development of the chick, to which reference has already been made. There seems to be little doubt that the chick embryo for a short time in its early development excretes 90 per cent. of its nitrogen as ammonia, then for a short time 90 per cent. as urea, and finally (for the greater part of the time) 90 per cent. as uric acid. Something of the same kind is seen in the case of the frog, for Bialasewicz and Mincovna⁽⁸⁾ found that 50 per cent. of the excreted nitrogen of the embryo was in the form of ammonia, but Przylecki, Opienska and Gedroyc⁽⁴²⁾ got only 12 per cent. in the post-metamorphosis stages.

In view of the fact (see (55)) that marine invertebrates excrete their waste-nitrogen mainly as ammonia; fishes, amphibia and probably chelonia mainly as urea; and sauria and birds mainly as uric acid, there can be no doubt that these are, in a sense, recapitulatory sequences. It is difficult, however, to imagine that the mechanism which produces the urea is essential as containing a formative stimulus for the mechanism which produces uric acid, although the correlation in time between maximum urea production and piscine morphological characteristics is close enough. It would be more convincing to suppose simply that just as in the phylogenetic order, urea excretion succeeded ammonia excretion, being one step more complicated, and in the same way uric acid succeeded urea; so the avian embryo, moving forward through its ontogeny in stages of ever increasing complexity and heterogeneity, excretes in the same order, ammonia, urea, and uric acid. Thus from this point of view the retention of ancestral organs and structures because of the formative stimuli which they provide, would become a special case of a general recapitulation theory. In other words, in Fig 1 the progress from Z_0 to A_0 would not be a kind of distorted image of the progress from A_1 to A_0 , but would retain from the lower parallel ontogeny lines (a) only such organs or structures as were necessary for morphogenesis, and (b) other general characteristics which belong necessarily to simplicity as opposed to complexity. The fertilised egg cell at Z_0 would not profitably be regarded as a recapitulation of the protozoan ancestor at Z_0 , but nevertheless there is an analogy, and in the same way it may be held that the ammonia-urea-uric acid sequence occurs in ontogeny simply for the same reason that it occurred in phylogeny, *i.e.* because it is a transition from the more simple to the more complex. The reason why the chick does not excrete uric acid from the very beginning would, therefore, be that it has not until a certain point developed the machinery for doing so, not that a urea stage was essential "physiogenetically."

Another instance of this is the appearance of hormones such as adrenalin, thyroxin, pituitrin, oestrin, and secretin in the developing embryo. As, to all intents and purposes, there are none in the unfertilised egg, and as invertebrates, especially the more primitive ones, are notoriously deficient in these substances (Riddle (44), Koller (27)) the recapitulation theory might easily be invoked. But it seems more reasonable to look on the two transitions, phylogenetic and ontogenetic, as passages from the simple to the complex, with all that that implies. This would be analogous with the case of the blastula which used to be regarded as a recapitulation of the coelenterate or other very early mode of existence. There seems no reason for laying much stress on such a correspondence, for a single cell, by dividing again and again could hardly do anything else, from the point of view of simple physics, than turn into a sphere or ball of cells, and that this should become hollow is more likely to be due to simple physical forces always acting in the same way than to the mysterious influence of a remote coelenterate ancestor.

Some workers have wished to regard the ash-content of animals as connected with recapitulation. In 1889 Bunge (10) discussing the significance of the occurrence, in the tissues of terrestrial vertebrates, of sodium chloride in quantities exceeding those of potassium and other elements, and the constant need for this salt in their

diet, advanced the view, strikingly novel for those days, that it could only be interpreted by the theory of evolution. It was natural that land vertebrates should maintain the relationship to salts which they had acquired earlier in their saline medium and this suggestion led in due course to the interesting work on the "palaeochemical endowments" of modern animals by McCallum (28). Bunge noted the relatively high ash content of embryos and emphasised that their sodium content declined with age; thus in the human embryo it falls (in per cent. of the total ash) from 25 to as little as 6 at term. This decrease in sodium was regarded by Bunge as due to the replacement of some of the tissues of more ancient origin by tissues developed to meet the requirements of the terrestrial adult, as for instance, cartilage by bone. It is doubtful whether these suggestions have much importance, at any rate, as yet, for we do not know enough about the ash content and the ash distribution in embryos all over the animal kingdom to criticise them suitably. In a case such as this, one might very well expect to find reflected in the constitution of the embryo the chemical characteristics of the temporary ancestral organs or structures retained on account of their essential stimuli, and if the sodium analogy means anything, it probably means this. But the declining amount of total ash would not be so explained, as there is no reason to suppose that the cells of the remote marine ancestor were richer in ash than those of later terrestrial organisms. As regards the appearance of palaeochemical ratios in the blood of terrestrial vertebrates, this hardly counts as an instance of recapitulation, for they do not copy their ancestors in development only, they copy them all the time.

It has also been suggested that the declining water content which all embryos exhibit during their ontogenesis is of recapitulatory significance. Tangl (50) who brought this notion forward, gave lists and tables showing the water content of various animals, but the number of investigations so condensed was rather small. It appeared from the data that the lower invertebrate forms had a higher water content on the whole, but the comparison involves some difficulty as the molluscan shells were probably not included in the estimations whereas the mammalian bones were. Unless one could be sure that the hard parts were not making the averages meaningless, Tangl's tables are not easy to interpret. And in any case, an association of high water content with recapitulation is not very attractive in view of the frequent observations of high water content in non-embryonic rapidly growing tissues, such as malignant neoplasms. The lipocytic coefficient (56) is closely associated with the falling water content during pre-natal life so that the latter is much more probably associated with high growth-rate than with any ancestral influence or characteristic.

A more interesting question is raised when the origin of the enzymes concerned in purine metabolism is considered, for Wells and Corper (53), Mendel and Mitchell (29), Jones and Austrian (25) and others found that during embryonic life the adult equipment of these factors is formed only gradually. Pig embryos less than 15 cm. long possess no nucleases, guanase appears first when a length of between 15 and 17 cm. is reached, and this is followed after an interval by adenase, while last of all, xanthineoxidase makes its appearance. There is thus some relation to the

phylogenetic order for Straughan and Jones (49) found only guanase in the yeast cell and no adenase, xanthineoxidase, or uricase, while in the mollusc *Sycotypus canaliculatus*, Mendel and Wells (50) found guanase and adenase but no xanthine-oxidase and no uricase. It would seem as if this is another instance of that progress towards greater complexity which we saw in the case of the nitrogen excretion of the chick, and we may assume that there is no fundamental reason why all the enzymes should not arise at once. They do not do so because the progress is a gradual one. But it is difficult to explain why they should arise in the mammalian embryo in the same order as they arose phylogenetically, for there is nothing obviously "simpler" or more "primitive" about guanase than there is about xanthineoxidase. Still, in this sequence nothing is lost once it has appeared, and the embryo as it becomes more complicated is only following out individually what the whole of living nature had previously done collectively. The work on these enzymes was continued by Przylecki and Rogalski (43), who made the very interesting discovery that in the early stages of the chick's development there is no xanthineoxidase present (this might have been expected from the work already mentioned), but also that uricase is present and afterwards disappears. The formation and excretion of uric acid coincide pretty well with this change, so that before it begins the chick can destroy uric acid but cannot make it from purine bases, while after it has begun the chick can make it from purine bases but has no power of destroying it. However, this is not the point of main interest, for we know that the purine bases are not the main source of the avian uric acid; it is more probably formed from the ammonia of protein breakdown by way of tartronic acid (Tomita and Takahashi (52)). What requires explanation here is the appearance of an enzyme in early development which afterwards disappears, an enzyme, too, characteristic of lower forms, e.g. Actinia, echinoderms, selachians, amphibians. It seems very difficult to suppose that any question of formative stimulus can be involved here, and the reflection that, as far as we can tell, the uricase has nothing whatever to do, adds weight to the suggestion that it appears as a by-product (linkage, correlated variation, like yellow α with curly γ) of the gill clefts or other piscine structures, which, we believe, are essential for other reasons. But a good deal more work is required before this question can be regarded as settled.

The lower animals have no power, broadly speaking, of maintaining a constancy in their internal environment. Similarly, the early stages of embryonic life show a lack of this power of independence. As regards osmotic pressure, the classical work of Backmann and Runnström (3), Przylecki (41) and Adolph (1) on the frog may be mentioned, and Bialasewicz (7) on the chick. It is true that Backmann and Runnström considered only the egg as a whole, but this criticism does not apply to Adolph's conclusions. And there is no doubt that the power of regulating heat production develops at a late stage of embryonic life, as for instance, is shown by the work of Pembrey, Gordon, and Warren (39), and of Brody and Henderson (9) on the chick. The chick reacts to temperature change as a poikilotherm until near the end of incubation. Giaja (19), too, has found that in the rabbit the thermogenetic margin increases constantly with age. No doubt this gradual attainment of freedom from

subjection to the inorganic environment only repeats itself ontogenetically for the same reason as the gradual increase in complexity of nitrogen excretion. That homoiotherms have to begin by being poikilotherms probably does not mean that the mechanisms involved in the latter condition exert any formative stimulus upon those involved in the former, but simply that to be a homoiotherm implies greater complexity and degree of adjustment than to be a poikilotherm. The ancestral stage is thus only present because each ontogeny has to begin again at the beginning and a homoiothermic egg cell is not a practical possibility. One might say that at a certain level of complexity poikilothermicity disappears or can disappear, so that a line drawn from $g-g$ in Fig. 1 would have poikilothermicity and low degree of organisation on the one side, and homoiothermicity and high degree of organisation on the other. The animals A_1 to A_4 would never succeed in gaining their independence of the environmental temperature, while A_5 to A_9 would do so. And it is very interesting that, just as increasing independence of the external world as to heat and osmotic pressure has shown itself in the phylogenetic series, so as regards the eggs themselves the tendency has been to make them ever more and more self-contained and immune from the fickleness of the inorganic realm. Precisely parallel with heat and osmotic pressure goes resistance to water loss, and just as the embryos of the highest groups begin by showing the same reactions as the adults of the lowest groups, so terrestrial animals, though fit for life on land when adult, are not so in the embryonic condition and are provided with amniotic cavities, "private ponds" in which to develop.

But perhaps the most striking instance of what happens when lower is transmuted into higher organisation, is the change in energetic efficiency which occurs as the chick develops. It has been shown (57) that although the average value for the whole of incubation is 66·5 per cent. (that is to say, out of 100 calories present in the yolk and white the chick can store 66·5 but must combust 33·5) the value is different for each day calculated separately and gives a series of points which lie on a smoothly ascending curve. Thus on the 4th day of development the efficiency is low (43 per cent.) and on the 20th day very high (70 per cent.), in other words the earlier in development the more wasteful the chick is, and greater frugality accompanies greater organisation. Now, as is well known, bacteria and yeasts not only have a very high energy turnover (fermenting in an hour many hundreds of times their own weight of substrate) but their efficiency is also very low. Thus for autotrophic organisms Becking and Parks (58) found values ranging between 5 and 15 per cent., for Nitrobacter in particular Meyerhof (59) got 4·5 per cent. and in Rubner's well-known experiments (60) the figures vary between 12 and 30 per cent. Stephenson (61), in her recent book, enquires whether these low efficiencies may not be due simply to an inability of these organisms to keep their enzymes apart from their substrates. "One may regard the evolution of a metazoal organism," she writes, "as invoking a process whereby the energy liberated in chemical activity, which in a microbe runs to waste, is so *organised* and disciplined that it is liberated when and where it can subserve function; apart from this it is cut down to a minimum." We may perhaps regard, then, the steady rise in efficiency which the developing chick exhibits, as an

index of its ascending organisation, and hence as a perfect example of the fundamental transition from simplicity to complexity (with all that that implies) which underlies all recapitulation phenomena. Specialisation of function may be a factor in this, and no doubt we cannot expect the early avian embryo to be as efficient as it later becomes, since the machinery for dealing with special departments such as excretion and respiration, does not develop until some time has gone by. It must be added, however, that an alternative explanation of the rise in efficiency is quite tenable, namely that the composition of the embryonic body on the fourth day is quantitatively more unlike that of its food than is the case on the eighteenth or nineteenth day; and the influence of this fact may be considerable.

It has also been suggested that some recapitulatory significance may attach to the general succession of energy sources during ontogenesis which has been observed by many workers, and to the succession of carbohydrate-protein-fat in the constitution of the embryonic body. In the chick embryo, for instance, the relationships could be expressed by saying that the order of importance in time runs thus: water → solid, inorganic substance → organic substance, carbohydrate → protein, protein → fat. The ontogenetic procession of entities comes out equally clearly if attention is concentrated on the points in time at which the individual entities are at a maximum of quantity or activity in relation to themselves. To suppose that the carbohydrate ever exceeds the protein, for instance, in absolute amount, or the solids the water, would be to misunderstand this way of considering the embryo. It is also true that there is a relation of simultaneity between combustion and constitution, maximum intensity of combustion seems to coincide with maximum structural importance. In considering these questions Murray (34) drew attention to certain facts which can be summed up thus: (1) Carbohydrate-protein-fat is the order of ascending calorific value. (2) Carbohydrate-protein-fat is the order of descending respiratory quotient, *i.e.* of degree of intra-molecular oxygenation. (3) The ratios carbohydrate:protein and protein:fat decrease in the standard diet of peoples as one passes towards the equator. (4) The simplest syntheses deriving their energy from solar radiation involve first the formation of low members of the carbohydrate series. (5) The corresponding importance of carbohydrates in the plant kingdom may be related to its geologically temporal seniority. (6) Nutritive material passing down the intestinal tract of the higher animals is attacked in the order carbohydrate-protein-fat in striking regularity. (7) Its absorption there follows the same order.

To these the following considerations were added (38): (8) Carbohydrate-protein-fat is the order of increasing molecular polarity, *i.e.* the effects of molecular orientation at surfaces become most marked in that succession. (9) Carbohydrate-protein-fat is the order of decreasing value of the molecule considered as a hydrogen-donator. (10) Carbohydrate-protein-fat is the order of decreasing solubility in pure water. (11) Carbohydrate-protein-fat is the order of decomposition when a tissue is breaking down in autolysis.

These are some of the ways in which the order holds. Probably not all of them are very significant and some may be perhaps illusory, but yet when they are regarded

together there is a curious sense of the presence of some fundamental but elusive relationship at which as yet we can only guess. In any case it is difficult not to be impressed with the way in which, during the development of the chick from its fertilised egg cell, there is a transition of importance from water to solid, ash to organic bodies, sugar to protein, and protein to fat. One cannot avoid noticing a similarity between this succession and that occurring in the evolution of life as a whole, a similarity strangely reminiscent of the ancient concept of the microcosm, recently reviewed by Meyer⁽³¹⁾. But many workers will prefer to dispense with these speculations, replacing them with more immediate causes. Thus the succession of carbohydrate-protein-fat seen in the sequence of combustion during avian embryonic life might be explained by some concept such as that of "ease of combustion" and it is possible that a less complicated mechanism is required to combust carbohydrate than to combust protein and so on. Thus the glucose molecule merely requires activation, the amino-acid molecule has to be deaminated before its carbon chain can be combusted, and the fatty acid has to be desaturated. It would not be difficult to work out some parallel explanation for the sequence of substances seen in the constitution of the embryonic body.

But this disquisition has led far away from the theory of recapitulation as ordinarily understood. It is interesting to recall that the facts which we class under the term "recapitulation" have been regarded by some thinkers, e.g. Bertalanffy⁽⁶⁾ as the supreme stumbling-block of any chemical embryology. But on the whole, we may conclude that there is more likelihood of recapitulation being explained by physico-chemical causes than of anything in physico-chemical embryology being explained by the theory of recapitulation. As an explanation itself, it is not at all attractive, for it can do no more than appeal to a mysterious and enigmatic force of heredity or evolutionary urge, and cannot explain why the nose of an ancestral portrait should repeat itself through endless subsequent generations of portraits, while all the other features fall into oblivion. On this view, "Oblivion blindly scattereth her poppy," as Sir Thomas Browne said, but if we accept the theory of formative stimuli outlined in the preceding pages, then the phenomena fall into a reasonable order, embryos only recapitulate what they must, and in a word, recapitulation is itself explained by the physico-chemical requirements of the developing organism.

SUMMARY.

A discussion of the transitory functions of embryonic life introduces an account of the theory of recapitulation as it stands at the present time. The similarity between organiser phenomena and the formative stimuli provided by recapitulated structures is pointed out, and it is concluded that recapitulation itself cannot be regarded as an explanation of anything. A survey is given of the chemical events in embryonic development which seem to possess recapitulatory significance. Recapitulation may be regarded as fundamentally the result of the necessary passage from simplicity to complexity, from low to high organisation, which is entailed by the metazoal sexual system of reproduction, with its single egg cell. The retention of visible organs or

structures from lower ontogenies in a given ontogeny is only a special case of this general rule and probably depends on the presence in them of essential formative stimuli.

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VIRUS DISEASES IN PLANTS

I. TRANSLOCATION WITHIN THE PLANT.

II. THE AMOEBOID INTRACELLULAR INCLUSIONS.

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(Received January 22, 1930.)

I. TRANSLOCATION WITHIN THE PLANT.

IN the great majority of cases virus diseases in plants are systemic. There are exceptions to this rule: cases are recorded in which symptoms have developed on an inoculated leaf without a general invasion (Priode⁽⁴⁾, Kunkel⁽²⁷⁾). But as a rule successful inoculation at any localised area, e.g. the tip of a leaf, is followed by the spread of the virus throughout the whole plant. It may not produce symptoms everywhere, e.g. in a leaf fully mature at the time of infection signs may never develop although the leaf contains considerable amounts of active virus. Nor is the distribution uniform: even in the one leaf areas may remain apparently unaffected in juxtaposition with areas showing pronounced signs, and those green areas have been shown to contain less virus than the obviously diseased portions (Iwanowski⁽²⁵⁾, Holmes⁽²²⁾). Further, there is sometimes a definite localisation of the virus within the plant. The present writer has found that in *Datura stramonium*, inoculated in a leaf with tomato yellow mosaic, the virus usually localises itself in a very few isolated and often remote parts of the plant, while the intervening portions contain none, although the virus must have passed through them. Such cases, are, however, exceptional and systemic distribution is the general rule.

Very little is known of the mechanism by which this spread is effected. The general outline of its progress has been followed by Böning in tomato and tobacco⁽⁸⁾ and cf. Doolittle⁽¹²⁾ in the case of cucumber). On entering the stem the virus travels along and enters each leaf in turn on its way, while continuing its advance along the stem. Within each leaf it advances from the base towards the tip, a process which takes considerable time, so that the next or several following leaves may be entered before it reaches the tip of the leaf first invaded. The spread occurs in similar fashion in the stem whether inoculation is made at the base or at the top, and within the leaf it may advance either acropetally, or basipetally, as is evident from the general development of symptoms when a single leaf tip is inoculated.

There would seem to be at least two obvious routes along which this spread may be effected. The virus may pass from cell to cell by diffusion. That such a passage is possible is certain, at least for certain viruses. The breaking of a few trichomes with an infected camel-hair brush lightly stroked on the leaf is enough to cause

transmission of tobacco mosaic (McKinney⁽³⁴⁾), and here the virus must pass through the cell walls to reach leaf tissues; and it is known that in such cases the virus is taken up almost instantaneously (Holmes⁽²³⁾). The liquid exuded from hydathodes in an infected plant usually contains virus, and in most cases this involves passage through a protoplast. We do not know how quickly such a diffusion can occur, but it would seem on general grounds that the rate must be slow in the case of agents which can be held back by membranes sufficiently dense⁽³⁹⁾, and it seems improbable, though one can hardly, in the lack of evidence, say it is impossible, that such rates as are detailed below could be attained by this route alone.

The other obvious possibility is that dissemination can take place through the vascular bundles, either in the xylem or the phloem or both. Clinical evidence strongly suggests that this does occur. In many diseases one of the earliest signs of invasion in a leaf is a clearing of the veins, a lightening of the colour and increased translucency. This is a characteristic sign, for example, in the curly top disease of sugar beet, in which disease knots on the veins are also characteristic of the more advanced stages. In the very different disease, mosaic of tobacco or tomato, it is common under certain conditions to see the veins picked out as a yellow network on the green background of the leaf; and similar appearances occur in aster yellows and in the mosaics of *Vicia fabia* (Böning⁽⁶⁾), lettuce, dahlia (Brandenburg⁽¹⁰⁾), beetroot (Böning⁽⁷⁾) and other plants. We have, however, no direct evidence whether transmission in such cases takes place in the xylem or in the phloem, and can only draw inferences from the course of development after inoculation and from the available evidence of the rate of spread.

Beijerinck⁽³⁾ pointed out long ago that, if a tobacco plant is inoculated with mosaic on one side of the stem, the first leaf to show signs was the one situated directly above the wound. The second leaf to show signs might be the next leaf in the orthostichy, after which no relation to the phyllotaxis was detectable, and indeed as a rule the lateral spread was evident before the signs appeared on the second vertically situated leaf. Beijerinck, following the opinion current at the time, thought that transmission must occur in the phloem, since the virus passed centripetally along the petioles from an inoculated leaf, but this argument, like some others, has lost its cogency to-day. Miss Goldstein⁽¹⁸⁾, who has studied with great elaboration the development of signs in the mosaic tobacco plant, also finds that the first leaf to show unequivocal symptoms is always situated above the inoculated leaf, but she found no regularity in the intervals between the two leaves, since the critical leaf might be the fifth, sixth, seventh or eighth leaf above, usually the seventh. Such experiments would indicate that normally the virus on entering the stem proceeded upwards in the plant; but caution is necessary in drawing conclusions from the time of development of symptoms. The younger a leaf at the time of infection, the more conspicuous usually are the signs and the earlier do they become manifest, and, naturally, the higher leaf is the younger. Holmes⁽²²⁾, however, relying not on symptoms but on the actual presence of virus as demonstrated by inoculation into other plants, found that in young tobacco plants the virus travels from the inoculated leaf only upwards at first, and does not appear in lower

leaves until three weeks later, i.e. long after its appearance in the upper leaves. This is not the experience of Böning⁽⁸⁾. He found, also in tobacco (presumably of a different variety and certainly of larger size), that on testing the tips of the leaves immediately above and immediately below the inoculated leaf, the virus was first found sometimes in the upper and sometimes in the lower leaf, but that both leaves showed symptoms on the same day. It would seem, then, that in general virus moves in either direction in the leaf, and that in the stem, even if the upward route is the more usual—which is not certainly established—downward transmission can on occasions occur at least as rapidly, and movement in both directions may occur in the same plant at the same time. This does not help us very much. We know that movement in normal phloem may proceed in either direction, and even in the transpiration stream movement downwards may occur, though perhaps more slowly, in the same plant at the same time as the general movement upwards (Snow⁽⁵²⁾, of the stimulant substance in *Mimosa pudica*). More definite indications are given by the rates of movement.

Allard⁽¹⁾ found that 3 days elapsed before mosaic virus, inoculated at the tip of a tobacco leaf, entered the stem. Böning⁽⁸⁾ also found in the case of the tobacco leaf that at least 2 days, sometimes 3 or 4 days, were occupied in travelling a distance of 13 cm. in the basipetal direction, and acropetal movement within the leaf took place at rates of approximately the same order. In tomato the movement was rather slower within the leaf, e.g. 9 cm. in 3 days for the mosaic virus. In the stem of tomato 4 days were necessary for traverse of 12 cm., 4 to 5 days for 20 cm. in the basipetal direction, and 3 to 4 days for 12 cm., 4 to 5 days for 25 cm. acropetally. He thought that the rate tended to increase as the virus progressed; and he made the observation that the rate was slower for the streak virus than for the virus of simple mosaic.

These rates are comparatively slow, viz. about 0·125 to 0·27 cm. per hour, but rates as slow or even slower are recorded elsewhere in the literature. McCubbin and Smith⁽³³⁾ grew tomatoes with lateral branches which were caused to root in separate pots, thus producing daughter colonies. When the secondary plants were well established, the parents were inoculated with mosaic, and at intervals thereafter the connections with the daughters were severed. Subsequent development showed whether the infection had passed over to the daughter plants. One might expect that transference by such a method would be exceptionally slow, but it was found that on the average the rate was about 0·1 to 0·2 cm. an hour. Murphy and McKay⁽⁴⁰⁾ found that in the potato over 8 but less than 14 days were required for leaf roll to pass from a graft 29 cm. above ground-level to the tubers, and as it took 10 days for the leaf roll to reach the third shoot (out of a total of seven) below the graft, the spread through the remainder of the stem must have occurred at a rate of the same order as those already reported.

Bennett⁽⁴⁾ in raspberries found that when a well-grown shoot was infected with curl, other shoots of similar size from the same root were not infected during the same season; and in peach yellows it sometimes happens that a branch remains free from disease when the other branches are demonstrably infected and perhaps

showing symptoms (5). In sugar beet it took 6 to 10 days for infection with curly top to spread from the outer to the inner leaves, and when infection was made on the inner leaves the virus was not found in the outer leaves after 6, 8, or 10 days, the plants having 10 to 14 leaves in all (Severin (48)). In small beet seedlings the times were shorter, e.g. from inner to outer leaves in 2 days, from outer to inner leaves in 4 days. In large cucumber plants Doolittle obtained a higher rate, viz. 21 inches in 12 hours, or about 4·5 cm. an hour. The rates vary somewhat with the conditions of the experiments. In a leaf already mature, for example, movement is slower than in a leaf young or vigorously growing; and excessive nitrogen manuring may increase the rate in such a plant as the tomato (Böning (8)).

All these rates are very slow, if transmission is by carriage in the transpiration current. Sachs (46) found that in most potted plants movement in the xylem took place at from 18 to 210 cm. an hour; in *Bryonia* and cucumber the rate might be 600 cm. Snow obtained rates of over 1100 cm. an hour acropetally in *Mimosa*, and Bose (9) rates much higher still. The movement can be slowed very markedly by checking transpiration, e.g. in humid atmospheres and in other ways (see Bose) but in the virus experiments recorded above the plants, although mostly grown in glasshouses, were almost all young plants in very active growth and transpiration may be presumed to have been proceeding without exceptional difficulty. It is true that we cannot expect small particles, such as virus may be, to travel at the same rate as water or even dyes, but even so the differences in the rates seem very large.

Only two instances are recorded in which the rates approximate to those considered normal for the transpiration current. Severin (48) fed leaf hoppers (*Eutettix tenella*) infected with curly top virus upon the leaves of sugar beet, and ascertained the time at which uninfected hoppers placed upon the petioles took up the virus. He found that in different experiments the virus travelled along the petiole $3\frac{1}{4}$ inches in half an hour, $3\frac{1}{2}$ inches in one hour (twice), $4\frac{3}{4}$ and $5\frac{1}{2}$ inches in one hour, the rates increasing somewhat with the temperature. The greatest rate observed was 7 inches in half an hour, or 36 cm. per hour. Storey (54) in similar experiments with the streak of maize obtained a rate of 20 cm. an hour on three occasions, and sometimes rates of about half this magnitude. Storey also found that the movement was not markedly delayed by cutting out the midrib of the leaf or cutting across the half-lamina on which the inoculation was made: as had also been found by Allard (2). In the experiments of both Severin and Storey inoculation was effected by insects, whose habit is to insert their stylets into the cells of the phloem, and it is possible that in these cases the inoculum was delivered directly into the conducting elements.

It is generally agreed that movement in the phloem must be slower than movement in the transpiration current, and the speeds quoted above would therefore seem to be more in accordance with phloem transport. There is little precise information as to the actual rate of movement in phloem and no certainty as to the mechanism by which that movement is effected. It seems clear that it cannot be by simple diffusion alone, and that its speed varies at different times and with the food requirements of different parts of the plant. Mason and Maskell (37) in the cotton plant found that translocation of carbohydrates took place at rates of 0·383

to 0.526 cm. per hour if the whole bark was taken as carrying, and 2.175 to 2.99 cm. if movement occurred in the sieve tubes alone. Such rates, if they can be taken as indicating the order of speed to be expected in general, approximate more to the rates of virus movement, but they too are rather high.

There is definite evidence in certain virus diseases that the phloem is involved. The galls of Fiji disease are always produced in phloem tissue. In the curly top of sugar beet the phloem undergoes a necrosis so marked as to be visible macroscopically on section of the beet. In leaf roll of potato a similar phloem-necrosis is a prominent feature, and it is recorded also in the brown bast disease of rubber (it is, however, not yet conclusively established that this is a form of virus disease). In these diseases there is excessive accumulation of starch in the diseased leaves, and, although there is a difference of opinion as to the cause of this accumulation and no agreement whether the necrosis in the phloem is primary or secondary to the accumulation, it is evident that the normal transport of carbohydrate from the leaf is greatly interfered with. Starch accumulation is found in other diseases also where there is no obvious involvement of the phloem, e.g. in peach yellows (5), in spinach mosaic (55), in tobacco mosaic (according to Woods (56) and Hunger (24), though Freiburg (17) and Dickson (11) do not agree).

Bennett (4) studied the movement of virus in raspberries infected with curl, and came to the conclusion that transport took place in the channels which conveyed elaborated food material. If a healthy cane were inoculated, any new shoots which arose subsequently from the same root came up infected, showing that the virus had reached the root; but canes which were already well grown at the time of inoculation did not normally develop the disease the same season. They could be made to develop it by cutting them back, and always in the following season the new canes were infected. He supposed that normally the canes already well grown did not draw on the root for nutrition other than water and minerals but made an adequate supply for themselves; the virus in the root, therefore, did not reach them. When cut back, however, and also in the new spring growth, they were forced to draw on the store of food in the root, and the virus moved with the food supply. If a cane were girdled completely, virus did not pass the girdle in either direction, but if even a small bridge were left across the girdle, the virus could and did pass across. The woody nature of the raspberry makes it a very suitable plant for such experiments.

It must be recognised that taken together the evidence is as yet not very convincing, and any inference from it must be provisional. But, as far as it goes, we may conclude that, except perhaps in special instances, virus does not travel in the transpiration current. It can, and does, pass from cell to cell, but the rates of movement observed make it improbable that this is the only method by which spread is effected. In most cases dissemination takes place through the phloem. It should be possible, one would imagine, to obtain more satisfactory evidence than is yet available. If a branch or branches were caused to transpire vigorously, while in the remaining branches transpiration was checked, the rates of movement of the virus in the two groups should give a good indication whether the xylem stream

took any part in the transport. Similarly controlling the direction of the phloem movement (as by defoliation, removal of axillary buds), and comparing the virus movement when the movement in the phloem was upwards or downwards, should give more direct information on the part played by the phloem. Such experiments, however, have not yet been carried out in detail, but investigations on similar lines are known to be in progress.

SUMMARY.

Virus diseases in plants are usually systemic, but we have little knowledge of the mechanism or route by which the virus spreads through the plant from the point of inoculation. Such spread can certainly occur by movement from cell to cell, and this may be the chief route, but the rate of spread seems high for such a process. On the other hand, it is low for transport by the water stream. The probability is that transport is mainly effected by the phloem, for which there is contributory support in clinical phenomena. The available evidence, which is mostly indirect, is collected, summarised and discussed.

II. THE AMOEBOID INTRACELLULAR INCLUSIONS.

The appearance of abnormal inclusions within the cell is one of the characteristic phenomena associated with virus. They are found in the virus diseases of man and various lower animals, including fish and insects, and are common in the diseases of plants. They are not found in all virus diseases, but when they do occur their appearance is so regular as to be in some cases a valuable aid to diagnosis, *e.g.* in rabies. In plants they are practically always extra nuclear: only one solitary instance has yet been recorded of an intranuclear inclusion (by Goldstein⁽¹⁹⁾ in a cell of mosaic dahlia). Their production is dependent on the nature of the virus rather than on the nature of the host plant. The virus of tobacco mosaic, for example, produces them in every host in which it produces symptoms, but cucumber mosaic never causes them, even in the same range of hosts and even when conspicuous symptoms are produced (Hoggan⁽²⁰⁾).

In normal plants it is usual to find cellular inclusions of various kinds, *e.g.* crystals, raphids, storage products, oil droplets, tannin masses, etc. These occasionally assume suggestive forms, and have been mistaken in virus-infected plants for flagellates or other protozoa, to which the disease has been attributed (Nelson⁽⁴²⁾). In virus disease, especially in the Solanaceae, it is usual to find substances in the cells which, though abnormal in shape, appear to be similar in nature to those which may be found in the cells of normal plants—*e.g.* the so-called striate material found in the cells of mosaic tobacco. With these we shall not deal here: they are fully described by Klebahn⁽²⁶⁾, Goldstein⁽¹⁸⁾, Iwanowski⁽²⁵⁾ and others; and there is general agreement that, although some of them may be characteristic of virus, they are certainly inanimate and products of the reaction of the cell to the presence of the virus. Yet other intracellular elements have been described as connected with virus disease, some of them granular, some more definitely characterised^(16, 38, 43, 53); with these also we shall not deal. In addition to all those, however, there is found

an inclusion of a different character, about whose nature there is at present no agreement, and it is with the intracellular body of this type that we are concerned here.

This is a mass of material which is not unlike protoplasm in appearance, granular or very finely reticulate in structure, and usually staining more or less deeply with aniline dyes. It is usually well defined, and sometimes appears to have a definite membrane or bounding-wall. In shape it is rounded, often roughly spherical but frequently elongated and the larger forms may be constricted or bent or of almost any outline, while still retaining the rounded contours wherever the body has any considerable mass. Typically, the body is vacuolate, with sometimes only one large vacuole, sometimes as many as ten or more small vacuoles, of which all may be scattered through its substance or some may lie at the periphery; the margins of the vacuoles often stain more deeply than the rest of the mass. Inside each vacuole may sometimes be seen a small particle with radiating threads; and in the substance of the body is regularly, though not invariably, a varying number of small deeply staining rounded or angular particles. The whole may have a size which varies in different cases from about 3 to 4μ up to 20 or 25μ in diameter, and in the elongated forms sizes of 35μ or upwards in the longer axis are recorded. There may be only one in a cell, and this is perhaps the most usual number, but there may be as many as ten and eleven bodies and these perhaps of different sizes, inside a single cell. They tend to be associated in position with the nucleus, lying in close contact with it, sometimes enveloping it: but they are never incorporated with it and when carried in the protoplasmic streaming may be seen to impinge on it, sometimes denting it temporarily and sometimes being indented by it.

Inclusions of this type (which have been conveniently named X-bodies by Miss Goldstein) occur in virus diseases of very different character and apparently never in the absence of virus. They are found in monocotyledons, e.g. sugar-cane, corn, Hippeastrum, winter wheat, and in dicotyledons, e.g. tobacco, petunia, potato, dahlia, cabbage. They have been most frequently described in diseases of the mosaic type, but are found in other classes of virus disease also, e.g. the Fiji disease of maize (Kunkel (28)), spike disease of sandal (Narasimhan (41)), while in some diseases, e.g. leaf roll of potato, they have not yet been seen. Minor differences are recorded in the various hosts and diseases, but there is no doubt that in all the cases they are structures of the same nature. They may occur in every tissue of the plant, including root and flowers; and are often curiously local in distribution, e.g. they may be present in almost every cell of a particular area, but absent in the immediate neighbourhood in the same tissue, and several microscopic fields may be passed in review in succession before another area is found where they are again almost universally present. As a rule, they are most common in the chlorotic areas but may be met with in regions which have no obvious disease and may be absent in areas which are markedly yellow. They are not artefacts: they are easily seen in the living cells; and it is quite clear that they are not distorted or degenerate forms of any of the organised structures of the normal cell, e.g. they are not degenerate nuclei or plastids, nor are they aggregates of tannin, resin or any similar material.

Their general resemblance to amoebae is undeniable and was pointed out in 1903 by Iwanowski who first described them. The resemblance is increased by the not infrequent occurrence of protuberances from the sides, often more hyaline than the general mass, which suggest pseudopodia; and some observers have not hesitated to conclude that they are certainly organisms more or less akin to amoebae, and have created new genera or species to receive them, and given them names, e.g. *Phytamoeba* (McWhorter⁽³⁶⁾), *Northiella sacchari* (Lyon⁽³²⁾), *Vacuolarium Iwanowski* (Likhite⁽³¹⁾).

There is considerable evidence that they increase in size. Indeed, it is difficult to imagine how it could be otherwise. Whatever their nature may be, they are certainly the result of infection of the plant with a principle small enough to pass through very fine filters and they develop within the cells as the result of its invasion by this minute agent or its toxins. In the early stages of the disease they are very small, but are larger in the more advanced stages (Kunkel⁽²⁷⁾: maize mosaic). In the growing point of mosaic tobacco they are small in the cells near the tip and larger in the larger cells of the young leaf primordia; and in the stem they were found to be larger in the larger cells underneath the epiderm than in the epidermal cells, and in the sixth or seventh layers, where the cells are very large, the X-bodies are also very large and sometimes enormous (Goldstein⁽¹⁸⁾, and cf. McKinney, Eckerson and Webb⁽³⁵⁾). As a general rule, when the process of their formation is complete, the larger bodies are contained in the larger cells. Rawlins and Johnson⁽⁴⁵⁾ think that the degree of development of the body depends more on the stage of the development of the leaf at the time of infection than on the interval which has elapsed since infection; which is, perhaps, another way of saying the same thing.

There is no direct evidence of autonomous movement. The bodies move freely within the cell in the protoplasmic streaming, but no motion has been observed which may not be merely passive, and the statement that autonomous motion does occur (Likhite⁽³¹⁾) is an inference from the appearances which suggest pseudopodia and not an observed fact. No nucleus or nuclear material has been demonstrated in them: the small deeply staining granules do not react like nuclear chromatin (Holmes⁽²¹⁾). They contain mitochondria in moderate numbers, and this fact is definitely in favour of the view that they are made up of protoplasmic material, whether of cellular or parasitic origin. (See infra, on their protein nature.)

It is not infrequent to see bodies which have a well-marked constriction near their middle, and from the examination of a large number of examples it is possible to arrange a consecutive series, which shows at the one end a single rounded body, at the other end two separate bodies, and in between these extremes every gradation of constriction from slight dimpling to a nearly complete constriction in which the two halves are still joined by only a slender band of connecting material. All the stages of a process of simple fission can thus be found, and evidence of this kind has led Kunkel and Goldstein to believe that X-bodies do in fact divide as an actual process of multiplication. Goldstein has also seen that in dividing cells it is usual for both the daughter cells to contain X-bodies, and is inclined to think that in the cellular division the bodies take up special positions in relation to the dividing

nucleus and themselves divide when the cell divides. Both these observers, therefore, think that X-bodies are alive, and Likhite is convinced of it. It may be noted that the constriction is not always near the middle, but may be near the end, that more than one constriction is not uncommon, and that it is possible to arrange similar series in which the ultimate separate bodies are of very unequal size—*i.e.* the process would be more akin to budding than to ordinary fission. In the tissues, further, where cells are dividing, there are often several bodies in each cell, whether it is dividing or not, and it is to be expected that the daughter cells would share them.

Summarised briefly, the evidence in favour of their living, independent or parasitic nature consists in (1) the general amoeba-like appearance, with which may be included the pseudopodium-like projections, which suggest amoeboid movement; (2) the appearances suggesting fission, and the occurrence of several bodies in the same cell, which may be due to division; (3) the increase in size; (4) their protein nature and the presence of mitochondria. It is not very convincing, and probably most workers incline to the view that these bodies are to be interpreted as a reaction of the infected cell to the presence of the virus.

According to Dufrenoy (13, 14, 15) the first sign of virus in a plant cell is a fragmentation of the vacuole. Normally the cell contains a single large central vacuole, and the cytoplasm is arranged as a peripheral layer along the wall. On the entrance of virus this is changed. The cell becomes penetrated in all parts by trabeculae of cytoplasm, between which are small vacuoles which may be rounded or filamentous, or of all shapes. The cytoplasmic strands are rich in mitochondria which may be short near the rounded vacuole or long and filamentous with the ends of adjacent elements apparently united together. The mitochondria are in active division: and many of them, especially in leaves infected young, evolve into amyloplasts; only a few chloroplasts develop and these imperfectly. This first stage is a stage of excitation. (Kunkel also states that in the mosaic of maize there appears to be a preliminary stage of excitation.) Similar phenomena occur in many plant cells under more or less normal conditions, where changes in the metabolic activity of the plant are reflected in changes in the vacuome, which may pass from the large single liquid area of the classical vacuole into the semi-fluid tiny filamentous, reticular or rounded forms or may transform into nearly solid masses (*e.g.* aleurone grains).

This stage is followed by a stage of mitochondrial fragmentation and vesication. Mitochondria are composed of a mixture of lipoid and protein elements; the plastids also normally contain lipoid granules. In degenerative conditions, these granules may increase in size and number, until the whole plastid, or sometimes only one pole of it, is converted into a fatty mass. This is what occurs in virus disease. The mitochondria swell up and vesiculate, sometimes into quite large aqueous vesicles. The plastids become fatty and lose their chlorophyll and starch, the spaces occupied by the latter sometimes remaining as empty spaces for a time. The liquid in the vacuoles changes its character, becoming less acid and of lower osmotic concentration than in cells where the chlorophyll retains its colour, and it

is less stable, being readily precipitated by neutral red *intra vitam*. The cytoplasm beside the vacuoles takes on a capacity for staining more intensely, and finally contracts into a vacuolated mass which is attached by many filaments of cytoplasm to the cellulose membrane at the points of intercellular communication. Dufrenoy seems to think, though he does not explicitly say so, that X-bodies are simply portions of such chromatophilic cytoplasm which have formed into vacuolated masses. The whole process he considers to be that usual in cellular degeneration from any cause, whether bacterial, fungal or chemical, the special difference observable in virus diseases being the peculiar chromatophilia which the cytoplasm acquires in the neighbourhood of the Golgi apparatus, *i.e.* the vacuoles.

This view accords well in certain respects with some recent observations of Sheffield and Henderson Smith (49). They have been able to follow in individual living cells the development of X-bodies from their early beginnings to their complete formation. In the early stages of infection there appear in the streaming protoplasm of the cell tiny particles which are carried round the cell in the stream. As time goes on, the particles increase in size and in their course along the cytoplasmic strands they tend to hesitate or halt at the strand junctions, until adjustment of the strands and modification of the shape of the particles themselves enable them to proceed. With still further increase of size, the halts get longer; they may continue for an hour or two. During such a halt a particle may be caught up by another particle, and, when movement is resumed, the two may again separate or they may proceed as one mass. By successive increments larger masses are eventually formed, which are recognisable as X-bodies. There may be several such masses moving independently of one another, or they may coalesce to form one or more larger masses. These composite bodies may remain permanently in union, and in that case they seem to fuse, as it were, together into a more homogeneous whole, in which vacuolation can be observed; and even in quite small masses vacuoles are detectable. Sometimes, however, a composite mass may again separate into two or more constituent parts, which resume their separate movement. When this occurs, figures which look quite like fission figures may be seen, but there is no division in the sense of multiplication and the separated portions may again unite. Similarly, when a small mass breaks away from, or joins up with, a larger mass, appearances may be presented which simulate pseudopodia. No evidence of autonomous movement was obtained.

The nature of the small particles is still undetermined: they give the appearance of being foci where the cytoplasm has condensed or consolidated. The X-bodies were found to give all the usual protein-reactions (*e.g.* Millon, biuret, etc.), and showed, in the plant used, a marked tendency to crystallise out, either as protein crystals or in semi-crystalline forms with faces and angles on part only of their surface.

This mode of formation accounts for the evidence which has led to the belief that X-bodies are living creatures, *e.g.* the fission figures, the pseudopodia, the presence of several bodies in one cell and the increase in size, as well as the mitochondrial content. If it is confirmed by other workers, there would seem little

reason to believe that the X-bodies are themselves organisms or stages in the life-history of the virus parasite. They should rather be looked on as a reaction of the cell cytoplasm, and it may be that each of the tiny particles observed in the early stages is evidence of a localised reaction to a still ultra-microscopic particulate virus embedded in its centre.

SUMMARY.

In virus disease in plants, abnormal inclusions, found only in such disease, are frequently found inside the cells. Of these inclusions one type has characters which have led to the belief that it may be a living amoeba-like organism, a phase in the life-history of the virus parasite. This type, the "X-body," is fully described and the evidence for its parasitic nature set out. The alternative view is that these bodies are not living organisms but are a reaction-product of the cell to the virus irritant. This seems, on the whole, more probable, and is supported by some recent work in which formation of the bodies has been watched in the individual cell from their beginning to their completion, the method of formation accounting for the appearances which have suggested that the bodies are independent organisms.

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VISCOSITY OF PROTOPLASM AS DETERMINING THE RATE OF BIOLOGICAL REACTIONS

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(Received January 27, 1930.)

IN a recent paper Bělehrádek (1929) has put forward the theory that the temperature coefficients of biological reactions are only temperature coefficients of the viscosity of the reacting protoplasmic phases. This theory is founded on two main propositions:

1. The rate of chemical reactions in a heterogeneous system depends solely on the rate of diffusion of the reacting substances.

2. The rate of diffusion is strictly related to the viscosity of the medium according to the formula propounded by Sutherland, Einstein and von Smolukowski. This formula states that the coefficient of diffusion is inversely proportional to the viscosity of the medium.

Hence, protoplasm being a heterogeneous system, the rate of a protoplasmic reaction is determined by the viscosity, and consequently any change in the rate of the reaction with change of temperature is determined entirely by change in the viscosity of the medium¹.

Without expressing acquiescence in the first of these statements, against the general application of which considerable evidence might be adduced, I propose in this paper to examine the second of Bělehrádek's assumptions, namely, that the coefficient of diffusion of substances in a heterogeneous system such as protoplasm is determined by the viscosity.

A relation between diffusion of non-electrolytes and viscosity of the medium was obtained mathematically by Sutherland and Einstein independently in 1905. According to them,

$$D = \frac{RT}{N \cdot 6\pi\eta\rho} \cdot \frac{1}{r},$$

where D is the coefficient of diffusion, R the gas constant, T the absolute temperature, N the Avogadro constant, η the viscosity of the medium and r the radius of the diffusing molecules, assumed large in comparison with those of the medium.

¹ Bělehrádek speaks of the *viscosity of the reacting phases of the protoplasm* ("Par conséquent, dans tous les cas, où la vitesse de diffusion est le facteur limitant de la vitesse d'un processus physiologique, le coefficient thermique de ce processus n'indique que le coefficient thermique de la viscosité des phases réagissantes du protoplasma"). There seems to be some curious confusion here. By the reacting phases are presumably meant the reacting substances, that is, the substances whose diffusion rate is said to determine the rate of reaction. But the η of the Sutherland-Einstein-Smolukowski formula refers to the viscosity of the medium, not to that of the diffusing substances.

Where the particles are smaller the relation, according to Sutherland, approximates more nearly to

$$D = \frac{RT}{N} \cdot \frac{1}{4\pi\eta\rho}.$$

The formula obtained by von Smolukowski (1906), namely,

$$D = \frac{64}{27} \cdot \frac{RT}{N} \cdot \frac{1}{6\pi\eta\rho},$$

only differs from the Sutherland-Einstein formula in the value of the constant. According to both, other factors being equal, the coefficient of diffusion is inversely proportional to the viscosity of the medium.

Although determinations of the viscosity of solutions are numerous, there are few data with regard to the effect of the medium on the coefficient of diffusion. Öholm (1912), however, determined experimentally the diffusivity of potassium chloride in aqueous solutions of glycerol and sucrose of various concentrations. He found, for example, that the diffusivity of this salt was 1.535×10^{-5} C.G.S. units in pure water, but only 0.486×10^{-5} in a $1.5 M$ solution of sucrose, and 0.255×10^{-5} in a $2 M$ solution, at $18^\circ C$. According to data given in Landolt-Börnstein's *Physikalisch-chemische Tabellen* the viscosities in C.G.S. units of sucrose solutions of various concentrations at (approximately) $20^\circ C$. are as follows:

Table I. *Viscosities of sucrose solutions at $20^\circ C$.*

Concentration of solution (%)	Viscosity of solution in C.G.S. units
1	0.01031
5	0.01127
10	0.01322
20	0.01910
40	0.06004

The viscosity of a 44 per cent. solution at $18^\circ C$. is given as 0.0860. The viscosity of pure water at $18^\circ C$. is 0.01056 and at $20^\circ C$. 0.0100.

Hence, if the Sutherland-Einstein-Smolukowski relation holds in this case, the coefficient of diffusion of potassium chloride in a 44 per cent. solution of sucrose at $18^\circ C$. should be reduced from 1.535×10^{-5} , the value in the case of diffusion through water, to about 0.18×10^{-5} . But Öholm has shown that in a still higher concentration, 51.3 per cent., the coefficient of diffusion is only reduced to 0.486×10^{-5} . We may safely conclude that in solutions of cane sugar of this order of viscosity the coefficient of diffusion is not less than four times as great as the value given by calculation based on viscosity.

There seems no doubt that the divergence between the actual values of the coefficient of diffusion and those obtained by calculation from viscosity determinations increases with concentration, but there is no evidence that the formula holds at all exactly for dilute solutions of sugar, although the divergence is not so great. If we assume, which perhaps we have no right to do, a linear relation between co-

efficient of diffusion and concentration of the medium, we obtain for the coefficient of diffusion of potassium chloride in 5 per cent. sucrose at 18° C., using Öholm's data, a value of approximately $1 \cdot 435 \times 10^{-5}$. Using Öholm's value (1905) for the temperature coefficient of diffusion of potassium chloride, this would give a value for the coefficient of diffusion of potassium chloride in 5 per cent. sucrose at 20° C. of $1 \cdot 502 \times 10^{-5}$. The theoretical value calculated from the viscosity is $1 \cdot 424 \times 10^{-5}$. The difference between the two values is not great, but it must be remembered that the viscosity is not far removed from that of water, nor is the coefficient of diffusion much removed from that in water, so that the values are bound to be fairly close. For 10 per cent. sucrose the values for the diffusion coefficient of potassium chloride obtained by interpolation from the data of Öholm and by calculation from the viscosity are respectively $1 \cdot 40 \times 10^{-5}$ and $1 \cdot 21 \times 10^{-5}$. It seems, therefore, that the coefficient of diffusion is always greater than that given by calculations based on the viscosity of the medium, and that, as might be expected, the more viscous the medium the greater the divergence.

Similar results are given by a consideration of Öholm's data for the coefficient of diffusion of potassium chloride in solutions of glycerol and measurements of the viscosity of glycerol solutions. The observed data for a temperature of 18° C. are shown in the following table:

Table II. *Viscosity of glycerol solutions and coefficient of diffusion of potassium chloride in such solutions.*

Concentration of glycerol (%)	Viscosity of solution	Diffusivity of KCl (Öholm) c.g.s. units $\times 10^5$	Diffusivity of KCl (calculated) c.g.s. units $\times 10^5$
0	0.01056	1.535	—
43	0.0432	—	0.375
51	—	0.501	0.228*
69	0.200	—	0.081
76.3	—	0.201	0.045*
81	0.732	—	—
86	0.971	—	—

* By interpolation.

While there are many determinations of the viscosity of suspensions and suspensoid sols, as far as I am aware determinations of the diffusivity of substances in such systems, with which the viscosity measurements can be compared, are wanting. With emulsoids we are, however, on surer ground. Bělehrádek quotes the work of Loeb on the effect of temperature on the viscosity of gelatin sols in support of his theory. Now we have a series of determinations of the viscosity of gelatin sols made by Bogue (1921) and quite a number of observations on the diffusion of substances in gelatin by different observers. These all indicate that the presence of the gelatin affects the rate of diffusion very little. In gelatin gels there is certainly a little retardation of diffusion, but Calugareanu and Henri (1901) concluded that certain organic dyes diffuse as rapidly through gelatin as through water if the gelatin had

not set to a gel. Exact measurements of the coefficient of diffusion of potassium chloride through gelatin of different concentrations were made by Öholm. In most cases he dealt with gels, but one of his observations was made with a 2 per cent. gelatin sol. In this case he found the coefficient of diffusion at 20° C. was reduced from 1.61×10^{-5} for water to 1.50×10^{-5} . Bogue found the viscosity for 2 per cent. gelatin 1.818 times that of pure water, which would give for the coefficient of diffusion of potassium chloride in gelatin at 20° C. a value of 0.89×10^{-5} . The calculated value, using the formula quoted by Bélehrádek, is therefore only 59 per cent. of the actual value. It is true that the viscosity measurements were made at 35° C. and the measurements of the coefficients of diffusion at 20° C., but this need not trouble us as the relation between temperature and diffusivity over the range 20°–35° C. may be taken as linear with approximately the same temperature coefficient for the different concentrations. There is, then, no evidence of any such relation between viscosity and diffusivity in the case of gelatin as that assumed by Bélehrádek.

But the most striking illustration of this lack of intimate relation between consistency of, and diffusivity in, colloidal systems, is afforded by gels of gelatin and agar-agar. The coefficients of diffusion of a number of substances in such gels of different concentrations have been determined by Stiles and Adair (1921), Stiles (1921, 1923) and Mann (1924), and it has been shown that the diffusivity of these substances is reduced only very slightly in gels below the diffusivity of the same substances in distilled water. A few of these observations dealing with sodium chloride are summarised in Table III. It will be observed that in a gel of 16 per cent. gelatin, a very stiff gel, the coefficient of diffusion is only 30 per cent. less than that of the same substance in distilled water. A very stiff agar-agar gel, for example, one of 4 per cent., only reduces the coefficient of diffusion by about 10 per cent. below that in water.

Table III. *Coefficients of diffusion of sodium chloride in gels of gelatin at 20° C.*

Concentration of gel (%)	Coefficient of diffusion in c.g.s. units $\times 10^5$
0	1.35 (by extrapolation) 1.31 (by extrapolation from Öholm's results)
2	1.29
4	1.25
8	1.14
16	0.94

According to Heilbrunn (1928) protoplasm is usually a suspensoid sol, but having regard to the substances such as proteins, fats and other lipoid substances which we know form part of it, it seems unlikely that the consistency of the protoplasm can be so simply defined, and for the purposes of the present discussion it

will be safest for us to regard the protoplasm as a complex colloidal system without closer definition, and in any case, when we find that the relation between viscosity of the medium and diffusivity is not that assumed by Bělehrádek, either when the medium is a solution of crystalloidal non-electrolyte or when it is an emulsoid sol or gel, it is most improbable that the relation should hold in the one case, that of suspensoids, for which the necessary data for settling the point are lacking. In any case, the onus of proof would rest on the propounder of the theory, who, however, quotes observations on gelatin, an emulsoid, in support of his views.

From this summary of the information available with regard to the diffusivity of substances in heterogeneous systems and its relation to the viscosity of the medium, we may conclude that in such systems the coefficient of diffusion is not inversely proportional to the viscosity. Actually the coefficient of diffusion is reduced relatively little for considerable increases in the viscosity of the system. This being so, we cannot suppose that the very considerable effect of temperature observed with many biological reactions is solely due to changes in the viscosity of the protoplasm affecting the rate of diffusion of the reacting substances.

Further evidence against Bělehrádek's theory is provided by observations on the effect of temperature on the viscosity of protoplasm. In *Amoeba* Heilbrunn (1929) has shown that with increasing temperature from 0° C. the viscosity remains approximately constant up to about 12° C. above which temperature it falls, reaching a minimum at 18° C. With further increase in temperature the viscosity again rises, reaching a maximum round about 25° C., above which temperature it again falls. A somewhat similar relation between viscosity and temperature is recorded by Heilbrunn (1924) for the protoplasm of the egg of the clam *Cumingia*.

Although there may be a few biological reactions which show a similar behaviour towards temperature (cf. Heilbrunn, 1928), such cases are certainly rare; indeed, as far as I know there is not a single well-authenticated case recorded for the whole of plant physiology.

It may be concluded then that the viscosity of the protoplasm, so far from determining the rate of biological reactions, generally plays a very small part, perhaps a negligible part, in determining the rate of such reactions.

SUMMARY.

A review of the available data shows that in solutions of non-electrolytes and in heterogeneous systems the coefficient of diffusion of substances is not inversely proportional to the viscosity of the medium. Consequently there is no basis for Bělehrádek's theory, based on such a supposed relation, that viscosity of the protoplasm, by determining rate of diffusion, determines the rate of biological reactions.

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BIOLOGICAL RACES IN INSECTS AND ALLIED GROUPS

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(Received February 4, 1930.)

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INTRODUCTION.

THE occurrence of what have been variously called biological, physiological or intra-specific races among insects has long been known. Recent work has however extended our knowledge of this subject to a great extent, and the close bearing that it has upon various problems of variation and evolution is becoming increasingly clear.

A proper understanding of the subject is obviously of enormous importance to the economic zoologist, but it is hoped that this review will indicate that applied entomology has a contribution of real value to bring to the study of problems of more academic interest.

A biological race may be said to exist where the individuals of a species can be divided into groups, usually isolated to some extent by food preferences, occurring in the same locality and showing definite differences in biology, but with corresponding structural differences, either few and inconstant, or completely absent.

It is of course well known that the phenomenon is found highly developed in organisms such as the Bacteria and Fungi where, owing to the relative simplicity of structure, morphological "characters" are few. In such groups the usual concept of a species (as a group of individuals distinguished from all other groups by the common possession of certain morphological characters) often breaks down and

merges insensibly into that of a biological race. In the bacteria in many cases it appears that even such structural characters as exist are actually less reliable than are the physiological ones (Reed, 1923).

In the animal kingdom the phenomenon crops up in many groups. In the Protozoa it is well known that certain Trypanosomes, forms of *Trypanosoma brucei* (Duke, 1921), for instance, which are practically indistinguishable but inhabit different hosts, can only be transferred with great difficulty and after a long period of culture on an artificial medium. There are many other instances among parasitic Flagellates but comparatively few have been so thoroughly investigated as has the case quoted above, and there is much confusion and much diversity of opinion on the subject (Chandler, 1923). Similar examples could be cited from the genus *Trichomonas* (Faust, 1921) and perhaps also among the Entamoebas, but a good deal more work is required before a decision as to the true status of the various forms can be arrived at.

In the Porifera the difficulty of finding reliable structural characters is so great that the definition of species is almost as great a difficulty as it is among the Bacteria. In the Coelenterata, while in many cases the troubles of the systematist are indeed acute, there is some hope that structures such as the gonophores and, in the case of the Actiniaria, the nematocysts will eventually yield good characters. In this connection the recent work of Stephenson (1929) is of considerable interest and seems worth mentioning here. In a number of species of Actinozoa, distinguishable in the live state by very slight but apparently constant differences in colour, structure, markings, etc., it was found that there were very great differences in the method of reproduction. Thus one species would reproduce by longitudinal fission, another by laceration by tearing, or by constriction, and yet another viviparously. Only one method prevails in any one species save that deposition of ova may coexist with any other method, except possibly in certain viviparous species. Other similar cases of great physiological differences between species structurally very closely allied have been recorded in other sedentary marine animals, e.g. the Ascidians (Berrill, 1928; see also Potts, 1910, p. 482). There can be little doubt that research specially directed towards this subject would yield interesting results in this and allied groups.

Instances may also be cited from the higher groups. Perhaps the most striking of these is that described by Cuénot (1917) in the different forms of *Sepia officinalis*. Three of these forms which are found in the same region have previously been considered distinct species, separable by size and by very slight differences in the form and structure of the internal shell; but Cuénot, after an exhaustive study of these, concludes that they are not sufficient to warrant specific distinction. He does however show that there is a well-marked difference in the reproductive and migration periods of these forms, resulting in a large measure of biological isolation, and accordingly looks upon them as species in the making. He says:

On peut concevoir que la première étape de la segmentation de l'espèce linéenne a été une différenciation éthologique, c'est-à-dire une sensibilité différente aux facteurs qui déterminent l'entrée en migration.

Cette différenciation... a produit une séparation physiologique apparemment complète, par maturation des œufs à des époques différentes.

La seiche nous fournit un nouvel exemple de ces espèces naissantes chez lesquelles la séparation physiologique et éthologique a dévancé de beaucoup la séparation morphologique encore peu ou point marquée.

Somewhat similar cases in which the chief differences are those of reproductive period are described in Vertebrates by Fatio (1888) in the fishes *Clupea* and *Coregonus*; and among the Amphibia by Boulenger (1898). (See Robson, 1928, p. 123.)

It is however when we come to the Arthropods and the Platyhelminths and Nematodes, groups which, in the former case especially, cannot as a whole be described as lacking in systematic "characters," that the occurrence of the phenomenon becomes of especial interest and it is accordingly to the Insects and certain other Arthropod groups and, to a lesser extent, to the Nematodes that it is proposed to devote the greater part of this review. To treat a larger field really adequately would be an immense undertaking, involving as it must almost the whole of the science of bacteriology and a great part of mycology and protozoology. Moreover, the cases which occur in the insects, mites and Nematodes almost all concern terrestrial parasitic or plant-feeding forms, which being somewhat similar in life history are conveniently treated together. With regard to the Cestodes little need be said, as it seems that no completely authenticated cases are known, although the work of Chandler (1922) and others on *Hymenolepis nana* of man and rodents, suggests that this may prove to be an instance. Dr Baylis however tells me that this appears to be a rather exceptional case and there is not at present much indication that the phenomenon is at all widespread among tapeworms.

HISTORICAL.

As far as insects are concerned the idea was first put forward as long ago as 1864 by D. B. Walsh, an American worker, who supposed, from his observations on various wood-boring and plant-feeding beetles, that races of this type, attached to different food plants, must exist. Walsh's work appears to have attracted little attention at the time of publication, and was totally neglected till Craighead drew attention to it in 1923. This neglect is all the more surprising considering that his paper appeared so soon after the publication of the *Origin of Species*. It reminds one of the similar neglect of Mendel's classical paper published the following year, 1865.

Walsh established two classes, phytophagous variety and phytophagous species, according to whether intercrossing takes place or not, and regarded the second class as equal in value to those species which can be distinguished on structural grounds. This arrangement cannot now be followed; the days are long past when sterility of hybrids was considered an infallible test of the validity of a species and few, if any, biological races are so strongly established that crossing *cannot* take place, although under natural conditions it may be extremely rare. Moreover, if we use the word "species" we should logically have to give them a specific name—an impossible proceeding; for what would be the state of systematic entomology, already disordered enough, if a large proportion of the species could be identified on breeding tests alone? If slight visible differences are present, insufficient and

too variable to warrant separation as a species, then a varietal name can be employed as *Pediculus humanus* race *capitis* (Nuttall, 1929). In some cases simple trinomials have been employed, as in the case of the biological races of *Pegomyia hyoscyami* (Hering, 1926 b, p. 132), in Aphids, and again *Ascaris lumbricoides suis* and *A. l. hominis* (Martin, 1926; see Sandground, 1929), but it seems advisable to reserve these for geographical races. To lump such a complex of forms together under one specific name is unscientific, and merely serves to hinder advance along biological lines; but at the same time some distinction should be made between geographical and biological races.

This of course does not necessarily imply that there is any fundamental difference between the idea of "physiological species" in Bacteria, and biological races in Arthropods, etc., on the one hand; and on the other, the meaning of the word "species" as generally used. In fact the present writer inclines to the view that there is not; in the one case morphological differentiation is uppermost, in the other physiological. In the bacteria the term "physiological species" is perfectly justified, since, in the group as a whole, morphological criteria are unreliable and, owing to the ease with which they can be cultured, investigation of the physiological reactions is quite possible. In the Arthropods it is not allowable merely because of its impracticability; and, moreover, it should be said that there are relatively few instances known in which a group of individuals so highly differentiated physiologically as to come within the concept of a species, yet lacks *all* constant differentiation of colour and structure.

Here, as in all discussions of this nature, we come up against the difficulty of finding a definition for the word "species," and as there is no universal criterion of specific status we are led to conclude that a species can only be defined as a group of individuals recognisable as such by that elusive person the "competent systematist"! In this connection a statement by Robson (1928, p. 18) seems singularly apposite. He says: "We have then to admit that there can be no 'species problem' in the sense of the discussion of the attributes of an entity having fixed boundaries and definite properties." But there is a species problem in the sense that the various groups recognised as such by the systematist, having different degrees of relationship, and differing from each other in diverse attributes, represent episodes in evolution." The chief interest of biological and geographical races lies in the consideration of them as representing episodes in evolution.

The close analogy between biological and geographical races will be seen from the definitions given above but, whereas the continuance of a geographical race is ensured by geographical isolation, the biological race is isolated by biological differences¹, such as food and egg-laying preferences, and by disinclination for cross-breeding. Indeed, in order fully to establish the existence of a biological race in a plant-feeding insect, for instance, it is necessary to show that the larvae

¹ The term "biological" is used here, and throughout this paper, in preference to "physiological" because of its wider connotation. In insects especially, many of the differences by which races can be distinguished are "psychological" rather than "physiological." Indeed, Thompson and Parker (1927) suggest that in many cases the selection of hosts by a parasite is a reaction *solely on the psychological plane*. (See also Forbes, 1909.)

of each form have a well-marked preference for the food plant of that particular race; that the adults have a preference for egg-laying on that plant and that there is a definite tendency for members of the same race to mate together rather than with individuals of another race.

At this point we should perhaps mention the "host selection principle" as it has been called, which has a close bearing on the subject in that it suggests a method whereby a large class of biological races may have been brought into being. A definition of the principle has been given by Hopkins (1917), in ignorance of Walsh's work. He says that "an insect species which breeds in two or more hosts, will prefer to continue to breed in the host to which it has become adapted." If this is so, then it follows that the species in question is to some extent, at least, differentiated into biological races.

For the sake of discussion it will be convenient to take firstly, cases in which the races occur naturally; and secondly, those in which they have been produced, or are supposed to have been produced, experimentally.

BIOLOGICAL RACES OCCURRING NATURALLY,

(a) Free living forms.

In the first group, one of the most interesting instances that has been observed is that of an Anthomyid Fly, *Pegomyia hyoscyami*, worked out by Cameron in England in 1914 and 1916. This insect attacks beet and mangold as well as other members of the Chenopodiaceae and Solanaceae, the larvae mining in the leaves. Cameron conducted experiments which showed that there were at least two biological races within the species, one confined to the plants of the order Chenopodiaceae and the other to the Solanaceae, and that within these two families preferences to different species might be shown. For instance, flies reared on mangold refused to lay eggs on the common Solanaceous weed, *Atropa belladonna*, and *vice versa*, and an experiment in which the two plants used were *A. belladonna* and *Hyoscyamus niger* (henbane) gave similar results. In some cases recourse would be had to the unaccustomed food plant only when no alternative was supplied, while in other experiments the egg-laying preferences were so strong that the flies refused under any conditions to lay eggs upon the food plant of the other race. A point of special interest is that there is a colour variety of the fly known as var. *betae*, and it is this form which seems especially concerned with the Chenopodiaceae. The evidence, therefore, suggests that in this instance the biological races are well established, and that structural differences have begun to manifest themselves.

As far as it goes this work appears quite convincing. It was carried out on a large scale in big outdoor cages. Hering (1926 b) also regards these forms as true biological races, and states (p. 137) that the genitalia do not yield any satisfactory distinguishing characters which would justify their separation as distinct species. It is, however, unfortunate that Cameron's work could not have been continued over a number of years to determine the effect of host plant change on the oviposition reactions in the second and third generations. It seems worthy of note that the larvae of these flies, being leaf miners, are in a particularly close relation

to their food plant. The intimate physiological adaptation shown by such leaf-mining larvae to the conditions obtaining in the particular tissues of the host plant in which they live has been demonstrated by Hering (1926 b). It is a point of importance in connection with the study of biological races.

Another interesting case in the Diptera⁽¹⁾ is that of the Apple Fly, *Rhagoletis pomonella* (Tryptidae), in the eastern United States. This insect has been very thoroughly studied owing to its great economic importance, the larvae being very destructive through their habit of mining in the fruits. Consequently there is a considerable literature dealing with it. It seems a clear case of a single species which is represented by two distinct forms or races, occurring on two different food plants in the same area, showing so far as can be discovered (Porter, 1928; Curran, 1924) no morphological differences whatever except for a quite constant difference in size. The insect seems without doubt to be a native of North America and its original host was apparently *Crataegus*. The larger form (Patch and Woods, 1922) now infests apple and related fruits and the smaller form blueberry and huckleberry. *Crataegus* is now very seldom attacked, and crab apple has only been recorded as a host plant very rarely. The average body length in the two cases is: male 4.60 mm. and 3.60 mm., and female 5.80 mm. and 4.20 mm., and although there is a considerable amount of variation, the two do not overlap. The two forms now seem quite separate biologically; neither race will oviposit on the food plant of the other, and it has been found impossible to transfer the half grown larvae of the blueberry form into apples, or *vice versa*, although it does not appear to have been recorded on how large a scale these experiments were carried out. There are also slight differences in behaviour between the two forms—the smaller race being much more active and wary than the other. Woods (1915) believes as a result of these observations "that biologically at least there are two distinct strains or races of *R. pomonella*, the one breeding in apple and related fruits and the other in smaller fruits such as the huckleberry and blueberry." He continues: "Certainly in so far as *Rhagoletis* occurs in Maine the form on the apple and the form on the blueberry are entirely independent. The oldest inhabitant of the barrens cannot remember a time when there were not maggots in the blueberries, while the introduction and spread of the apple maggot in the State is a matter of record.... In Maine the blueberry maggot apparently did not migrate to the apple nor *vice versa* and the two races have lived on independently side by side."

In the Orthoptera there is one very striking instance known, and without doubt a great many others await discovery. Fulton (1925), in a study of variation in the Snowy Tree Cricket (*Oecanthus niveus*), found that in Oregon there are two races which differ only in their habits. Race "A" inhabits trees and is practically identical with individuals of the same species from the eastern States, the eggs being placed singly in the bark of prune and apple. Race "B" is confined to bushes in Oregon, and the eggs are laid in compact rows in the *pith* of shrubs such as loganberry and wild raspberry. The females of each form select plants for egg-laying which best meet the requirements of their characteristic mode of oviposition, and this appears to be the main factor in determining the choice of

environment. Study of a large series failed to reveal any pigmentary or morphological characters by which the two forms could be separated, but they can be distinguished in the field by very well-marked differences in song, race A having a frequency of notes almost twice as great as race B. Not only were races A and B constantly different in song but both were distinct from the eastern form. The characteristic song and oviposition habits remain fixed when adults of one form are confined to the normal environment of the other, and it is difficult and in some cases impossible to induce A to lay eggs on shrubs or B on trees. Experiments suggest that there is a mating repugnance between the two forms, but much more work is necessary before this can be considered definitely established. The same author describes similar races of *Nemobius fasciatus* in Iowa, inhabiting different ecological niches and quite indistinguishable morphologically although easily separable by song; but this species was not studied in such detail.

Among phytophagous Coleoptera a suggestive instance is that supplied by the work of Craighead (1921) on various Cerambycidae, the larvae of which bore in wood. For these experiments eleven species of beetles belonging to eight different genera were employed, some being monophagous, others oligophagous and others attacking a considerable variety of hosts. The work was carried on over a number of years, the insects being given their choice of cut logs of different species of host plant under as natural conditions as possible in large outdoor insectaries. As far as possible, allowance was made for the variation in the optimum conditions of the wood required by the different species, and also wood from several individual trees was used so as to avoid the possibility of offering an undesirable sample.

The conclusions reached by Craighead are so striking that it seems advisable to recount certain of his experiments in some detail.

Xylotrechus colonus is a polyphagous species which has been recorded from nearly all the hardwood deciduous trees of the eastern and central U.S.A. It shows little or no preference for any exact condition of the wood, save that it will not attack perfectly seasoned material. The original strain obtained for the experiments came from oak and showed a decided preference for a few woods—especially oak, chestnut and hickory. Two years' trial failed to produce larvae capable of completing their development in locust, while the ash and maple colonies were maintained with difficulty. Originally the oak strain showed little preference as between oak, hickory and chestnut; yet, after a period of from 4 to 6 years, strains were developed in each wood that showed a growing preference for the given wood. It was also shown that when few insects are present they concentrated on the original or favourite hosts; when more than could successfully develop in the original hosts are present, less favoured hosts were taken.

Similar experiments were conducted with two strains of *Cyllene pictus*, one attached to *Hickoria*, the other to wild grape, *Vitis*. Although in the naturally occurring races the host preference is well marked, when the insects are placed in somewhat overcrowded cages, oviposition on woods other than the primary host could be obtained without much difficulty, and the resulting larvae developed with ease. In this way a mulberry (*Morus*) feeding race was developed from the hickory

strain which, in two years, showed a very striking preference for the new host plant. It was also shown that there is a tendency for individuals to mate with like forms rather than with members of the other race, although unfortunately no figures are given to show the strength of this tendency.

Perhaps the most interesting experiments carried out by Craighead were those with *Callidium antennatum*, a species feeding only in pine and spruce. A strain obtained from pine failed to reproduce in spruce; one or two eggs were laid on the latter but no larvae succeeded in completing their development. When, however, half-grown larvae were transferred to spruce wood, although a considerable mortality resulted, a number came through with the result that a strain was produced which showed a decided preference for spruce over pine. Unfortunately no figures are given, the author merely stating that the spruce was well infested.

The same tendency was shown to exist in varying degrees in the other species studied, and from the observations of Hopkins (1917) it appears highly probable that the phenomenon exists in the Scolytid beetle *Dendroctonus monticolae*.

In evaluating such experiments as these it must be borne in mind that there are many factors involved, such as age and state of seasoning of the wood, which may very greatly influence the results and which cannot altogether be eliminated however carefully the experiments are conducted. These experiments seem to show that well-developed biological races of these various wood-boring beetles do exist in nature. They also suggest that continued breeding in a certain host over a number of generations intensifies the predilection for that particular host; but the figures given are not sufficient, nor were the controls extensive enough, to enable one to come to any definite conclusions on this point, nor as to the method by which the new strains were produced under experimental conditions. From the nature of the case it would be very difficult to obtain an accurate record of the number of eggs laid to compare with the number of larvae maturing in each host, and no such attempt was made, although an accurate knowledge of the mortality during development in the original host, and after a host plant change, would be most valuable. Craighead also concluded that the fewer the number of hosts a species has in nature the greater the predilection for a particular host. Here again the evidence, while suggestive, does not appear sufficiently extensive to permit of a generalisation. That the same tendency is also developed among other Cerambycidae in other regions of the world seems highly probable. A peculiarly striking case, possibly of a similar nature, which recently came under the notice of the writer is that of the genus *Plagithmysus* in the Hawaiian Isles. There are three species, *P. darwiniatus*, *P. lamarckianus* and *P. varians*. Although very closely related and even though all three may occur within an area of a few square yards, each keeps to its own food plant and they are said not to mix or interbreed. To quote Dr Perkins (1899, p. xvii): "It is hardly conceivable that species can be more closely allied than these and yet remain distinct." An experimental study of such forms would be of the greatest interest, and might throw light on the extraordinary orgy of "speciation" which is such a well-known characteristic of oceanic islands. Mating experiments in particular are desirable.

Before leaving the Coleoptera one might also mention the clear case of a Flea Beetle, *Haltica bimarginata* (Woods, 1917), one strain of which readily feeds upon *Populus balsamifera*, while the other refuses it completely.

The present writer (1929 and 1930) has recently carried out some experiments on the small Ermine Moths (*Hyponomeuta*), which are at times such serious defoliating pests of apple and other fruits in Europe; the chief food plants of the species concerned being apple, hawthorn and blackthorn. The ground colour of the fore wings of the moths is very variable, all shades from dark grey to pure white being found. These colour forms appear to be correlated to a considerable extent with the food plants, the dark grey form being most frequent on hawthorn and blackthorn whilst the pure white form is predominant upon the apple. Careful investigation has failed to reveal any constant structural differences between these forms. Larvae taken from one plant would not willingly feed on any other but could be forced by starvation to adopt the food of the other race. When this was once accomplished they might show an actual preference for their new food, although those larvae reared on a strange plant generally emerged as rather undersized and often infertile moths.

In addition there are one or two interesting biological differences between the two forms. The cocoons of the apple-fed larvae are generally composed of a rather dense white silk, and the pupae are usually placed together in neatly arranged rows or packets. The hawthorn- and blackthorn-feeding larvae, on the other hand, as a rule only spin very flimsy silken cocoons through which the pupae can be easily seen, and these are very frequently scattered at random in the web, close packets being the exception. Again, a leaf-mining habit is usually present in the first larval stage of the apple feeder, but not in the case of the hawthorn form.

Table I gives the results of oviposition experiments carried out over a period of two years.

Table I. *Showing the approximate number of eggs laid by *Hyponomeuta padella* (hawthorn form) on the food plants.* (From Thorpe, 1929.)

Oviposition of *H. padella*. Hawthorn form.

Exp.	Hawthorn and blackthorn		Apple		Total	
	No. of batches	No. of eggs	No. of batches	No. of eggs	No. of batches	No. of eggs
1	11	476	6	192	17	668
2	4	86	0	0	4	86
3	11	349	1	45	12	394
Total	26	911 79·3%	7	237 20·7%	33	1148

Average number of eggs per batch on hawthorn, etc., 35·0; on apple, 33·9.

Larvae taken from hawthorn and blackthorn were reared in large numbers in cages on their normal food plant. As the moths emerged they were placed in large cages and given a choice of plants for egg-laying, the apple being rather in excess of

the two other plants together. Table II gives results of similar experiments with the apple form.

Table II. *Showing the approximate number of eggs laid by Hyponomeuta padella (apple form) on the two food plants. (From Thorpe, 1929.)*

Oviposition of *H. padella*. Apple form.

Exp.	Hawthorn and blackthorn		Apple		Total	
	No. of batches	No. of eggs	No. of batches	No. of eggs	No. of batches	No. of eggs
1	7	232	19	550	26	782
2	1	60	26	1520	27	1580
3	0	0	7	315	7	315
4	1	45	30	878	31	923
5	1	30	5	132	6	162
Total	10	367 9·75%	87	3395 90·25%	97	3762

Average number of eggs per batch on hawthorn, etc., 36·7; on apple, 39·0.

Experiments carried out with hawthorn- and blackthorn-reared moths, giving them a choice of these two food plants, indicated that the hawthorn-blackthorn race is subdivided in the same way. Thus, out of 825 eggs laid by the hawthorn-reared insects 18·8 per cent. were laid on blackthorn and 81·2 per cent. on hawthorn, whereas out of a total of 4293 eggs laid by the blackthorn form corresponding figures were 69 per cent. for the former plant and 31 per cent. for the latter.

Other tests in which equal numbers of typical males and females of the apple and hawthorn races were placed together in cages, all the moths of one race being marked so that they could be easily distinguished, showed that the number of like matings was roughly twice as great as the number of crosses, or in other words that the attraction between like forms was about twice as strong as that between unlike.

In contrast to *Hyponomeuta padella* it is interesting to note that a very closely allied species, *H. cognatella*, which feeds on a number of species of the genus *Euonymus*, shows no tendency whatever to form biological races. The original host of this insect is the deciduous *Euonymus europaeus*, but it is very common in gardens at Cambridge and elsewhere on the introduced evergreen, *E. japonicus*. The former is a relatively rare plant and is very much less frequent in gardens than is *E. japonicus*, so that the great majority of the *H. cognatella* must have been isolated on the latter plant for many generations. Yet experiments carried out during two years gave no suggestion that there has been any tendency to develop a race attached to that plant, the slight preference for oviposition on a deciduous *Euonymus* such as *europaeus* or *americana* having been retained unaltered.

There are many other points of interest in connection with this group of moths, such as problems concerned with genetics, the behaviour of the hybrids and the effect of host plant change over a number of generations, which the writer has not so far been able to investigate. It can, however, be said that host plant change over

one generation, while seeming to affect the oviposition reactions of the resulting moths to a certain extent, does not change them completely and consequently it seems improbable that they can be explained entirely on any mnemonic theory. More work must be done, however, before it is possible to generalise on this point.

(b) *Parasites.*

The cases so far considered have all been phytophagous forms. Some instances occurring among parasites will now be discussed.

Perhaps the most outstanding and well-authenticated case among insects of true biological races, as opposed to a "bio-geographical" race such as those discussed later in this review, is that of the Human Lice, *Pediculus capitis* and *P. corporis*. These two insects, the one infesting the human head and the other the clothing and body hair, were regarded by the majority of workers from the time of Latreille (1803) onwards as two distinct species. The detailed work of Nuttall (1919) however showed that while typical *capitis* and *corporis* are distinguishable, they represent the extremes of one species, *P. humanus*, and that they are identical in all essential points of structure; while Bacot (1917) showed that the hybrids were healthy and fertile to the F_3 generation. Nuttall also pointed out that the points in which the two differed might easily be accounted for by the effect of the different environment. Thus *corporis*, living in the clothing, takes large meals at relatively long intervals, whereas *capitis* feeds much more frequently. This habit of gorging, with the resulting increased internal pressure, would appear to explain the larger average size of *corporis*, especially the increase in size of the abdomen coupled with the loss of angularity in the abdominal segments, and the more widely separated hairs upon the abdominal surface. He continues: "the effect of darkness no doubt is responsible for *corporis* possessing larger and slimmer antennae and legs than *capitis*. The latter is more exposed to light upon the head than is *corporis* beneath the clothing in most instances. It is of course well known that Arthropods inhabiting dark places have longer antennae and legs than those living exposed to light."

Sikora (1917) was the first to bring forward evidence that the transference of *capitis* to the arm resulted, after four generations, in the production of a colony of typical *corporis*. As has been said above, the distinction of the two forms is not an easy matter, and Sikora in 1919 published a paper in which she said that her previous results were incorrect and founded upon an error in identification. In the meantime, however, similar experiments had been carried out by Bacot (1917; see also Nuttall, 1919) with the result that *P. capitis* bred for two years in boxes had mostly assumed the characters of *corporis*, while after three years they could under no circumstances be taken for anything but a pure strain of *corporis*. Unfortunately no data are given as to the initial mortality at the commencement of this environmental change, but there is no suggestion in the account that any great difficulty was experienced in making the transfer. The reverse experiment of rearing such modified *capitis* or typical *corporis* under normal conditions upon the head has not been carried out. Freund (1927) claims to have discovered differences in genitalia between the two forms.

Keilin and Nuttall (1919) bring forward evidence to show that the two strains

of lice invade each other's feeding grounds, and that there are ample opportunities in nature for the intermingling of *capitis* and *corporis*. Besides the slight structural and colour differences observable between these forms, there are also biological differences which are quite characteristic for the "average" of each form, and some of which, at least, are equally modified by change in environmental conditions. Thus *capitis* is more active at lower temperatures, it can climb more actively on hair, its fertility is somewhat lower under experimental conditions, and (Bacot) it prefers to lay eggs on hair, whereas *corporis* prefers to lay on cloth and, when laying on hair, does so awkwardly.

It has usually been assumed that *capitis* is the primitive form and that *corporis* was gradually evolved from it in response to the changed conditions, as man grew hairless and adopted clothing. Nuttall, however, in view of the ease with which *capitis* can be changed to *corporis* experimentally, says: "there is little doubt in my mind that *capitis* is being converted into *corporis* to-day in nature, and that the latter, when man has become hairless, will constitute a species whose birth we are now witnessing."

When we come to the Mites we are at once confronted with the great difficulty of finding reliable characters for systematic work. The differences generally used to distinguish "typical" individuals of the genus *Sarcoptes*, for instance, obtained from different hosts, are chiefly points of size, coloration, markings and chaetotaxy; characters which, with the doubtful exception of the last, are notoriously unreliable for specific differentiation. As a result of this the most generally accepted view was that there was one species, *Sarcoptes scabiei*, which gave rise to different races living in the skin of man and various animals; although of course there were also the "splitters," such as Berlese, who recognised fifteen species instead of one. Much of the earlier work was really quite insufficient to permit of any valid conclusions being arrived at, as Warburton's (1920) excellent review showed. Some of the "species" were based on descriptions as much as 70 years old, made with a technique and equipment quite inadequate for the proper study of such minute details, and often upon material quite insufficient for any estimation of the amount of variation occurring.

This view as to the specific identity of all these forms has, however, been on the whole confirmed by more recent work, especially that of Buxton (1921), who showed that there were no constant characters which could be used to differentiate the itch mites of the horse from those of man.

The experimental evidence as to the adaptability of the forms to different hosts is conflicting, and there is no case so well established as that of *Pediculus*. Cameron (1925) reports negative results in attempting to transfer the *Sarcoptes* of cattle to horse, sheep, rabbit, rat, cat and man, but the experiments do not appear to have been done on a very extensive scale. Similarly Shilston (1915) was unable, in spite of repeated and long-continued attempts, to infect either goats or rabbits with *Psoroptes communis* var. *ovis* from sheep; and Delorme (1926) reports transmission of the *Sarcoptes* of guinea-pigs to ferrets, but not to dogs, sheep, cows, pigs and horses.

These cases seem, however, to be rather exceptional. The great majority of

experiments on transmission result in a temporary establishment on the new host, but without the development of a new race. Thus, the transmission of mange from camels to man is by no means rare, and severe symptoms are produced (Cross, 1924), but there does not seem to be any authenticated instance of transmission of this severe form from one man to another. Similarly the *Psoroptes communis* of goats can be transferred to sheep and causes mange but dies out in the second generation, and the rabbit *Psoroptes* when established on sheep behaves in the same way. The status of the *Sarcoptes* causing Norwegian Crusted Scabies of man is in doubt, but according to da Costa Lima (1928) the rarity of this disease may be due to infection by a variety, of which a domestic animal is the usual host.

There are many cases on record of ready transmission of mites, particularly *Psoroptes* and *Sarcoptes*, from one host to another (see Hirst, 1922), but in no instance does there seem to be any adequate information as to structure and relative ease of transmission of the succeeding generations. There is clearly a big field for research, and one which promises results of great theoretical interest and importance.

How far the same phenomenon occurs among free-living mites is doubtful; our knowledge of the subject is as yet too meagre, but the valuable work of McGregor and Newcomer (1928) may be cited. It had been supposed previously that *Paratetranychus pilosus* attacking deciduous fruits, and the Citrus Mite, *P. citri*, were races of the same species. These workers, however, not only found very minute morphological differences which they regard as sufficiently constant to warrant specific separation, but also that while cross-mating takes place as freely and regularly as like-matings fertilisation never results, the offspring all being males (both species arrhenotokous). They also showed slight differences in habits, egg-laying and food preferences coupled with differences in geographical distribution.

On the other hand, we may also quote Tillyard (1929) with regard to *Tetranychus opuntiae*, introduced into Australia during the course of biological control work against the prickly pear: "It is proving to be one of the most destructive enemies of prickly pear known. Although morphologically inseparable from the common red spider of orchards and gardens, it is yet quite incapable of feeding on anything but *Opuntia* and is therefore a very important example of a physiological race which has been arbitrarily given a distinctive name."

Nalepa (1917), in a general discussion of the systematics of gall mites of the family Eriophyidae, took the view that while there are many observations which point strongly to the existence of races structurally identical but differing in the type of gall produced, yet certain proof is lacking in every case.

(c) Biological differentiation associated with geographical isolation.

Another very big group of cases is supplied by organisms, mainly insects, in which biological differentiation is associated with geographical isolation, but with little or nothing in the way of structural or pigmentary differences. Such populations are essentially geographical sub-species, in which the criteria of differentiation are biological. And just as the work of Pictet (1922) and Harrison (1920, etc.) has shown conclusively that in many cases the slight colour differences characteristic

of sub-species of Lepidoptera are germinally fixed and constant in a new environment¹, so it seems probable that slight biological characters will also prove to be germinal. There is already some work which points to this conclusion (see Harrison, 1920 and 1926), and it appears from the second paper that further investigation is likely to reveal matters of great interest in connection with the mode of inheritance of such characters as larval food preferences.

We can describe, firstly, a rather clearly defined set of instances in which the chief differences are concerned with the mode of reproduction in certain cases, associated with some difference in chromosome number or behaviour.

Parthenogenesis is well known in the Lepidoptera among the Psychidae, and the work of Seiler (1923) has shown that *Solenobia pineti* and *S. triquetrella* each exists in the form of two races, one parthenogenetic, the other sexual. In each case the two races occupy distinct, but overlapping areas. Similar, but less thoroughly studied instances are known in other Psychidae; and in Orthoptera (Myrmecophilinae, Phasmidae) (Vandel, 1928). Hering (1926c) cites the case of the Agromyzid Fly, *Phytomyza crassiseta*, and in the Hymenoptera, there is the well-known case of difference in incidence of worker production in the genus *Bombus* (*B. kirbyellus* and *B. hyperboreus*) in different areas.

The possibility of the occurrence of regional parthenogenesis in *Halictus* has been pointed out by Stöckhert (1923) and an instance is known in the Tenthredinid, *Blennocampa affinis* (see Vandel, 1928). There is a well-authenticated instance in the Thysanoptera (*Anaphothrips striatus* in North America) and, according to Vandel, similar cases probably await to be elucidated in Coleoptera (*Otiorrhynchus* and *Adoxus*). The biological differentiation of the races of the Honey Bee and the Silkworm, while of very great interest, is not discussed here, as these insects have been domesticated and selected by man over such a long period.

Many striking instances may be found among Coccidae, Aleyrodidae and other allied plant-feeding Homoptera. Thus in Europe and America *Trialeurodes vaporariorum*, the Greenhouse White Fly, has two forms, which differ in their parthenogenetic behaviour in that the English race gives rise to females (thus tending to become purely parthenogenetic) and the American to males (Williams, 1917; Schrader, 1926). The cytology of the English race has not been investigated in detail, but as regards the American race, the eggs that are not fertilised develop with the haploid number of chromosomes and produce males; those which are fertilised regain the diploid complex and give rise to females. It should be pointed out that an arrhenotokous race first recorded in England at Merton, Surrey (Williams), is increasing and spreading rapidly, but it is uncertain whether this arose *de novo* in this country or whether it was accidentally introduced from America. Dr Williams tells me that there is a possibility that the thelytokous race is a seasonal form found only in winter. That the two forms are one species morphologically seems established without any doubt.

A very remarkable case on the same lines is afforded by the recent work of Marchal (1927) on the Chalcid *Trichogramma*, the larvae of which are parasitic in

¹ The same has been shown with regard to sub-species of the Rodent *Peromyscus* (Sumner, 1923).

the eggs of a great variety of Lepidoptera. Marchal describes two forms of this insect occurring in the same locality in Paris, distinguishable only by slight colour differences but with great biological differences associated with different modes of reproduction. Typical *Trichogramma evanescens* has eight to ten generations a year consisting of both males and females, all of which are winged. Parthenogenesis results in males only. The other form, *T. cacaeciae*, which has different tropisms and oviposition reactions, usually has only two generations a year. The spring generation has only vestigial wings and reproduction is normally thelytokous, males being very rare. Although mating between *cacaeciae* ♀♀ and *evanescens* ♂♂ takes place freely, fertilisation does not seem to occur, as typical *cacaeciae* ♀♀ only result. The rate of development of the different forms is closely correlated with that of the host, but from his experiments the author concludes that this last characteristic is directly dependent upon external causes (phenotypic) and not genetic. There is also a thelytokous European race which is structurally identical with an arrhenotokous American form, *T. minutum* (*pretiosum* Ril.). There is no doubt that many similar instances await description in this widespread genus with its many so-called species, but before the status of these can be decided much work remains to be done. In the meantime they are perhaps best considered as well differentiated biological races. Marchal says (p. 523): "il est vraisemblable que si l'on poursuivait des observations dans la même voie, en faisant varier les origines tant au point de vue des stations que des hôtes naturels, on trouverait une longue série de lignées de Trichogrammes ayant la signification de races ou d'espèces élémentaires dont la parthénogénèse thélytoque aurait facilité la formation." (2)

Returning again to the plant-feeding Homoptera, there is the perplexing group *Aphidae*. According to Mordwilko (1923-7) many of the Aphids which reproduce by parthenogenesis only are not true species, but are biological phases of some sexual form temporarily absent from the area. It is supposed that when, during the glacial epochs, the winter host plant of a migrant species of Aphid died out in the more northerly parts of its range, the insect would be left marooned on a group of unrelated summer host plants and could only survive as a parthenogenetic ("anhocyclic") race of a species which might still exist as a "holocyclic" form elsewhere. Thus, according to Mordwilko, *Forda* on grasses in Northern Europe is really congeneric with the holocyclic *Pemphigella* whose winter host is *Pistacia*. Similarly, if it was the summer host plant which was destroyed, then the Aphid survived on the primary host and parthenogenesis would be lost. This, according to Mordwilko, explains the origin of the two forms of *Chermes abietis*, the one on larch and spruce in Western Europe and *C. a. abietis* on spruce alone in Russia, and likewise the similar life history of *Cnaphalodes strobilobius*, the larch form of which, previous to Börner's work (1908), was confused with that of *Chermes abietis*.

Mordwilko would also account for the origin of the similar races or species (see Steven, 1917) of *Chermes piceae* (*C. nusslini*) and *C. pini* (*C. pini* var. *orientalis*) (Marchal, 1913) in this way. In this latter case "spanandry" (scarcity of ♂♂) is a characteristic of *C. pini* *sensu stricto* (Western Europe) which is confined to *Pinus*

sylvestris, and which cannot now use the alternate host *Picea orientalis* even if present. The form from the Caucasus, *C. pini orientalis*, is holocyclic and if introduced to Western Europe still continues its full life cycle, provided *Picea orientalis* is present. As Wardle (1929, p. 9) has pointed out, there are several serious objections to this theory as a general explanation of specific differentiation in Aphids, some of which Mordwilko himself admits. For instance, it appears that a very high proportion of tropical Aphids are anholocyclic, and of these cases the hypothesis offers, of course, no explanation. Again, the theory presupposes that nutrition is the chief factor influencing the production of sexual forms, a point by no means established. More recently Mordwilko (1928) has extended and modified his theories to meet some of these objections.

The Vine Aphid, *Phylloxera*, owing to its great economic importance and its highly complex life history, has been more thoroughly studied than perhaps any other Aphid. It seems fairly well established from the work of Stellweg, Börner, Marchal (1923, *q.v.*) and many others, that there are different biological forms of this insect differing in the amount of injury to the host plant which they cause and responding in different ways to the varieties of vine, while morphological peculiarities which were at first thought to be diagnostic have since been proved unreliable (Börner, 1924). Thus a certain type of *Phylloxera*, owing to the absence or scarcity of American vines, will reproduce parthenogenetically for a number of years exclusively on European vines, and will thus become so modified as to be unable to resume its normal cycle on American vines. It appears probable that the so-called "Lorraine race" of *Phylloxera*, which is widely distributed in Europe and which appears sporadically in many countries, may have been developed by years of reproduction on European vines without the intervention of a sexual generation.

Lack of space prevents further discussion of this problem in Aphids, but it must be said that the instances given are merely a number of the better studied and more striking, selected from a host of examples which are probably of the same nature.

Where parthenogenesis is found among Crustacea many similar cases occur (*e.g.* Phyllopoda, Cladocera, Ostracoda, Isopoda). The well-known instance of *Artemia salina*, which has been thoroughly studied by Artom (1911), will serve as an example. Within the one morphological species of this animal two races, cytologically and biologically well defined, can be distinguished. One which is haploid in constitution develops by parthenogenesis only, the other which is diploid being sexual. A closely similar state of affairs is known to exist in *Daphnia pulex*.

Vandel (1928) has shown that the phenomenon of regional parthenogenesis is well developed in certain Chilopods and in Wood Lice of the genus *Trichoniscus*, and he takes the view that these and certain other cases illustrate the birth of new species, the differentiation of which is hastened by the isolation resulting from the parthenogenetic mode of reproduction.

We come now to another phase of the question of biological differentiation in geographical races, in which the distinguishing characters are of a different kind;

differences of reaction, physiological and psychological, rather than of reproductive methods. In this class there are many instances exhibited by insect parasites of insects, which have come to light as a result of recent work on biological control of pests, and the group Tachinidae is particularly interesting from this point of view. Thus in the United States there exists an insect originally named *Masicera myoidea*, but which it appears is impossible to distinguish by any morphological character from the European *Paraphorocera senilis* which attacks the larvae of the European Corn Borer, *Pyrausta nubilalis*. The natural host of *M. myoidea* is *Papaipema nitela*, and it does not attack the larvae of *Pyrausta nubilalis* which has recently been introduced into the region. According to Thompson and Parker (1927), even when the two hosts occur together on the same individual plant, only the *Papaipema* are attacked by the parasite. Again, the Tachinid, *Parexorista cheloniae*, is morphologically identical with *Carcelia laxifrons*, but the former in Europe attacks *Euproctis chrysorrhoea* while the latter in the United States parasitises *Clisiocampa* (Thompson, 1923). Another remarkable point with regard to *Paraphorocera senilis* is that this insect in Europe consists of at least three distinct races, almost if not quite indistinguishable in the adult stages, but having rather feeble but quite constant differences between the larvae (Thompson, 1922). One of these forms is a common parasite of *Pyrausta nubilalis* in the greater part of Europe, but in spite of very extensive data available as to the parasites of the Corn Borer, the other forms have never been encountered in this host. Similarly two other Tachinids, known under the one name *Pales pavida* Meig., and seemingly quite inseparable as adults, exhibit clear larval differences and are confined to different hosts, namely the Brown Tail Moth (*Euproctis chrysorrhoea*) and the Processionary Caterpillar (*Thaumetopoea (Cnethocampa) processionea*). Unfortunately details as to the mating preferences and results of hybridisation of these forms are lacking. They would be of the greatest interest and well worthy of study.

Instances such as these again bring us to the difficulties of the species problem, for if constant morphological difference between two forms is present in any stage we should, theoretically, recognise them as species, but to do so would in practice be impossible. This phenomenon (poecilogony of Giard) of species being inseparable as adults but distinguishable as larvae is, as far as is known, very rare among insects, although as larval taxonomy is developed, doubtless many more instances will be discovered. It may, as Thompson (1922) has pointed out, be regarded either as an example of the effect of different larval environments acting on one species and producing the beginnings of evolutionary divergence; or, focussing attention on the similarities of adults, as a case of convergence resulting from the action of similar environments upon forms originally unlike. Thompson, comparing the development of two such species to two mathematical curves such as the circle and "Pascal's snail" having different formulae but being practically identical in certain sections, argues that such instances could exist even were species as fixed and immutable as the mathematical formulae for the curves in question, and that they furnish no evidence either for convergent or divergent evolution. This view is no doubt valid from the purely theoretical standpoint, but it seems to the present

writer that the hypothesis of evolutionary divergence has the more to support it, for it is unlikely that such an extremely close resemblance could have been brought about by convergence, especially when we consider that the environments of the two forms are not absolutely identical.

To return to structurally *identical* forms. It must be borne in mind that there are three possible ways of explaining regional differences in behaviour of a parasitic species:

- (1) As the expression of the characteristics of germinally fixed biological races, *how induced does not immediately concern us*;
 - (2) As the direct effect of general environmental differences;
 - (3) As the direct effect of biological differences, either germinal or environmental, between the races of the *host* species in the two areas;
- while of course the possibility of all three acting together must not be overlooked.

With regard to *Trichogramma*, *Aleyrodes* and with certain similar instances described above, we can be practically certain that we are dealing with examples of the first and not the second class. But in no other instance have we sufficient knowledge of a parasite species to decide for certain to which of the three groups it properly belongs. The results of biological control work, however, suggest that as a rule the races of parasitic insects, when transferred to new areas, remain essentially unmodified, at any rate for a number of generations (*i.e.* are germinally fixed).

While there is no doubt that some instances properly fall under the second category we have no definite proof that this is so, although *Carcelia laxifrons*, mentioned above, may be an example.

This insect has, since its introduction into the United States in 1908, lost its habit of attacking *Euproctis chrysorrhoea*. This has been put down to hybridisation with the American race, and crossing between the two is known to take place, but Thompson suggests (1923) that it reacts to the new environment in the same way as the native form with which it exists side by side. Experiments show that the difference between the two races of the parasite lies in the oviposition reactions.

That biological races of a host species may exist, and may result in inability of a certain parasite to survive in a new area, has been clearly demonstrated by the work of Compere and Smith (1927), for while the Red Scale *Chrysomphalus aurantii* of the Orient is indistinguishable structurally from that of California, the Chalcid parasite, *Comperiella bifasciata*, which in the Orient attacks both *Chrysomphalus aurantii* and *C. aonidum* impartially, when introduced into California can flourish only on the latter. There appears to be no doubt that this is due to a physiological difference in the scale from the two regions, for Mr Compere tells me that while oviposition on *C. aurantii* will take place freely in California, the host always destroys the larvae by phagocytosis. The failure of the larva of *Tachina mella* to develop in *Liparis dispar* in the United States may be another instance of this.

Before leaving this phase of the subject there are one or two parallel instances which deserve mention. The first is Roubaud's theory (1920) of biological differentiation put forward to explain the absence of malaria from North-western Europe, despite the presence of potentially malarial mosquitoes. The theory postulates that

in certain species there is one race which prefers human beings and another adapted to feeding on domestic animals. There is now a voluminous literature on the subject, but Howard (1924 a) has published a useful summary of the state of affairs up to that time.

That the presence of domestic animals affords man a certain amount of protection from the bite of *Anopheles maculipennis* in many areas has been known for years and has been observed by a number of workers. It is stated by Roubaud (1920) and Wesenberg-Lund (1921) that *A. maculipennis* in Northern Europe is mainly a domestic insect, whereas south of the Alps it appears to be truly wild. It is also argued by Roubaud and others that the practice of keeping animals in cattle sheds, stables, and piggeries in the north has resulted in the development of a zoophilic race and that man is seldom or never bitten. The evidence for this conclusion is, however, by no means satisfactory. That there are two races of *A. maculipennis* occurring in Holland overlapping in geographical distribution and showing some correlation with the incidence of endemic malaria, seems to have been amply demonstrated by the work of de Buck (1926), van Thiel (1927) and de Buck, Swellengrebel and Schout (1927 and 1929). These races can be distinguished by average differences in size, wing length, number of maxillary teeth and colour, and it appears from the work of van Thiel (1927) that they are not entirely due to differences in amount and nature of food. There are biological differences correlated with these differences in form. Thus the larger form, which is more characteristic of uninhabited areas, hibernates completely, does not feed at all in the winter, and does not suck human blood readily in spring and early summer; whereas the other feeds impartially both in houses and stables at intervals throughout the winter and is said to prefer slightly brackish water for breeding. There is no difference in the infectibility of the two types by tertian *Plasmodium*. Hybridisation experiments unfortunately have not yet been carried out, nor have the mating preferences been tested.

These biological differences may possibly account for the incidence of malaria in this particular region, though even here there are certain anomalies. They do not, however, afford any support for the generalisations of Roubaud and Wesenberg-Lund; and, as the authors point out, there is no hope at present of explaining the phenomenon of "anophelism without malaria" the world over on any one theory. Experiments with two species of *Anopheles* in the United States have so far failed to show any similar division into biological races in that country (Bull and Root, 1923; Barber and Haynes, 1924).

The attempt to control the prickly pear (*Opuntia*) in Australia has brought to light an interesting case among the Coccidae. The Cochineal insect, *Dactylopius tomentosus*, consists of distinct biological strains specialised to different species of *Opuntia* and which, according to Hamlin (1926), can only adapt themselves with difficulty to others. These have been introduced into Queensland, and according to the latest reports appear to retain their specific reactions equally well in the new environment. Thus (Dodi, 1927) the race obtained from Chico, California, is destructive to *Opuntia inermis*, while a Texas strain favours *O. stricta*; both

strains are equally at home on *O. tomentosa*, as is also a third race procured from Arizona.

It is interesting to note that Plotnikov (1927) has recently brought forward evidence that the migration of Locusts is due, not as Uvarov (1921) supposed to alternations of migratory and non-migratory forms of one species, but to the presence of two very closely allied species (races?) in the same area; the one liable under certain conditions to form migrating hordes, the other always solitary. This of course is a modification of Uvarov's theory which, if substantiated, might justify us in regarding the migratory and non-migratory forms of many locusts as biological races of one species. According to Plotnikov, while under certain conditions each form may vary in the direction of the other, the two are essentially distinct so that one never actually gives rise to the other. The intermediate forms are regarded as hybrids. It seems doubtful, however, whether Plotnikov's data are as yet sufficient to justify the conclusions, and for the present at any rate Uvarov's version has more to support it.

Before leaving this phase of the subject, it is as well to point out that we are not really justified in considering forms such as have been just described as true biological races, unless it has been shown that when introduced into a new environment they are relatively stable. While there is plenty of evidence with regard to the first group which shows differences in the mode of reproduction, there is as yet little proof with regard to those which show differences of behaviour and host preference only, although the host preferences of the different biological strains of the Cochineal insect (*Dactylopius tomentosus*) introduced into Australia from the United States appear so far to have remained constant in their new environment; this is, however, offset by the case of *Carcelia laxifrons* which appears to be unstable in its new habitat. It is, however, to be hoped that in view of the great activity with which biological control work is now being carried on, a more definite answer to such questions will be forthcoming before very long.

Of course one could cite innumerable cases of slight biological differentiation in otherwise well-marked geographical races, but to do so would be outside the scope of this paper and, moreover, with one or two exceptions we have no means of knowing whether many of these differences, such as variations in breeding period, are the direct effect of climate and other environmental factors, or whether they are germinal.

It will be obvious to all who read this that the list of *probable* instances of biological races could be extended almost indefinitely. Every taxonomist will be able to think of likely cases in his own group, and the field for further research is boundless. A few particularly striking examples from different Orders which have come under the writer's notice in this country, and which would seem well suited for intensive experimental work, may be listed:

Lepidoptera.

The complex of British forms synonymised by Meyrick (1927 b) under the names *Nepticula aurella* St. and *N. poterii* St. and many other leaf miners.

The numerous varietal forms occurring in the genus *Eupithecia* (see Speyer, 1883).

Heteroptera.

The different forms of *Notonecta glauca*. These are now usually considered distinct species on the ground that intermediate forms are not found. Delcourt (1909) states, however, that there is a complete series of intermediate forms between *N. glauca* and *N. furcata*, and Poisson (1924) has described biological differences concerned with reactions to varying salinity of the medium. There also appears to be selective mating between these forms (Delcourt).

Homoptera.

The British species of the genus *Psylla*, with many closely allied species on different food plants.

Coleoptera.

The Chrysomelid *Adimonia capreae*, feeding on osiers and heath.

Many doubtful species of the leaf-mining Agromyzidae.

Hymenoptera.

Many British gall-making and other Tenthredinids of the genera *Pontania*, *Pteronidea*, *Trichiosoma*, etc.

Pompilids in which great variations of habits occur within a single species (see Bouvier, 1920; etc.).

A word of warning is perhaps necessary here. It is by no means suggested that the principle is of universal application, and it must not be expected that every such case will yield positive results. One or two negative results have been recorded after elaborate experiments, and these are sufficient to show that the principle is not of universal application.

Thus the experiments of Quayle (1926), while not completely conclusive, failed to show that the curious habit of the Codling Moth (*Carpocapsa pomonella*) of attacking walnuts in California was associated with the development of a biological race. Again, the extensive work of Thompson and Parker (1928) failed to show that individuals of *Pyrausta nubilalis*, the European Corn Borer reared from maize, were biologically differentiated from those on the original host plant, *Artemisia*; except that the slight positive attraction which *Artemisia* individuals have for oviposition on that plant seems to have been lost by the maize individuals.⁽³⁾

Again, the thorough and extensive work of Larson (1927) on the oviposition responses of the "Pea Weevil," *Bruchus quadrimaculatus*, led him to the conclusion that the adults of this species show no marked predilection for the host in which they have bred, and that continued breeding in a given host does not appear to intensify the preference for that host.⁽⁴⁾

(d) *Nematodes.*

The problem of host preference among the Nematodes is a big one and can only be dealt with in brief here. A very clear case is provided by plant-feeding species of the genus *Tylenchus*. *T. dipsaci* is notorious as a pest of strawberry and various other plants in the United States. In certain areas strawberry and narcissus are

the two main smallholding crops, and have been alternated on the same ground for many years. No case of attack on strawberry is ever seen, while heavy infestation of the bulbs is common. In another bulb area Hodson (1926) records that oat, a well-known host of this eelworm, is frequently grown in the vicinity of infested bulb fields, and even on ground which probably ceased to grow bulbs because of the depredations of this pest, yet no case of infestation of oats is ever seen. Yet again oat fields which are very severely attacked may contain clover, another favourite host, in flourishing condition; thus, in one instance, a field of "abundant" oats, so badly infested as to yield only 8 bushels per acre instead of 45, was followed in the autumn by a good crop of red clover which, according to Hodson, showed no sign of attack either then or subsequently. A set of field experiments confirmed these observations entirely, and further results of a similar nature have recently been recorded by Fox Wilson (1930).

An even more striking instance is supplied by the earlier work of Liebscher (1892) on *Heterodera schachtii*. A plot of ground was continually planted with peas for about thirteen years till it became so badly infested that the harvest amounted to only 4 per cent. of the quantity of seed used. Separated from this plot by a path 1 metre wide was a plot on which oats had been planted for seventeen years, which was also badly attacked and described as "oatsick." Both plots were then sown with a number of crop plants with the results graphically indicated in Table III. Liebscher, without going into minute details, assumed from these results that he was dealing with two different species, but this conclusion was almost certainly erroneous, for all the more recent work on these forms has led to the conclusion that there is no morphological distinction whatever. Steiner (1925), in an excellent review of the subject, explains Liebscher's results as follows. "The *Heterodera* populations on both plots were probably first identical, starting from the same infestation. Then the population on the pea plot lived for years, that is for a long series of generations, exclusively on peas and became highly specialised on this host. The population of the other plot, however, for at least seventeen years (*i.e.* for about seventy generations) lived on oats and had become highly specialised on this plant." Similar well-authenticated records of biological races of *H. schachtii* adapted to sugar beets, hops and barley are mentioned by Steiner, and this list could be very greatly extended. The work of Baunacke (1922), with the sugar beet form, showed that the animals were able actively to locate their favourite host at a considerable distance, moving through the soil of the experimental plots against the water flow, and that within a year from the commencement of the experiment they had spread a distance of 9 metres across the plot.

The original assumption was that these Nematode races, while not morphologically distinct, were fixed and constant biological races, and that the apparent increase in virulence of a strain reproducing on one host over a number of generations was due merely to the selection of a pure line from a composite population. There is, however, a growing body of evidence which indicates that this explanation is inadequate. In this connection Tischler's (1902) experiments with *Heterodera (Caconema) radicicola* are of particular interest. Working with a strain adapted to

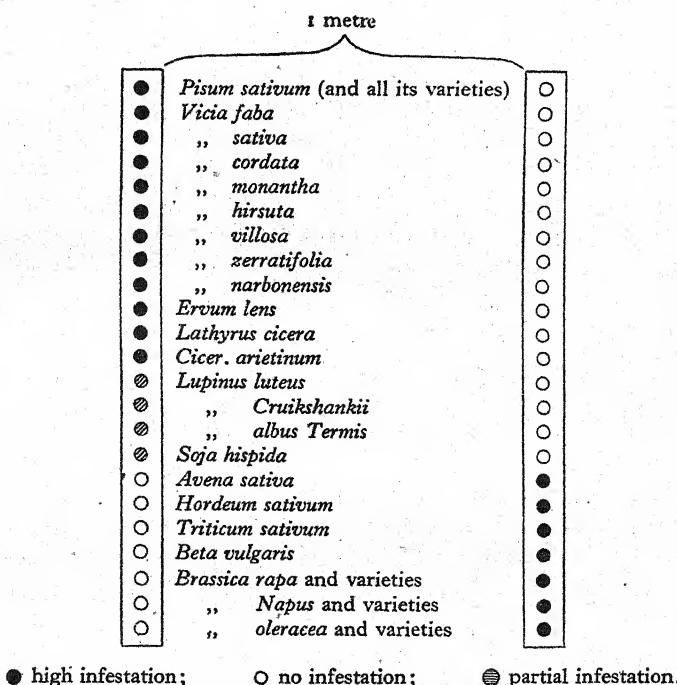
Circaeа lutetiana, it was found that even if *C. intermedia* be grown in such close proximity to *C. lutetiana* that the roots of the two intertwine, it remained *absolutely free* from infestation; if, however, the nematodes were given *only* *C. intermedia* they would infest it.

Hodson (1926) also records strains of *Tylenchus dipsaci* which, having developed for three or four years on narcissus, failed completely to establish themselves on oats or clover. Similar results were also obtained with onion, potato and oat strains.

Table III. Diagram to illustrate the experiments of Liebscher (1892) with *Heterodera schachtii*. (From Steiner, 1925.)

Plot planted for 13 years with peas and finally highly infested with a strain of *H. schachtii* specialised on peas

Plot planted for 17 years with oats and finally highly infested with a strain of *H. schachtii* specialised on oats



● high infestation;

○ no infestation;

○ partial infestation.

In some of these cases it was found that, although the nematodes did actually effect entry into another host, they were unable to feed and became sluggish and transparent, resembling individuals which had been kept in distilled water for long periods. While in some instances such individuals die out in a few weeks, in others they may persist for months, in which case (to quote Hodson) "they might be expected to adapt themselves eventually to a new host."

It seems that such results, particularly those of Tischler, cannot be explained entirely as due to isolation of pure lines.

To summarise, we may say that a plant-feeding Nematode prefers to feed on

the host species or variety upon which its parents have lived. This preference grows with the number of generations that a population lives on it, the Nematode thus getting more and more specialised to this particular host. The injury to the host from a specialised strain is correspondingly more serious than that caused by a generalised strain. "Finally the specialisation may reach such a high degree that even new hosts of the closest taxonomical, physiological and chemical relationship to the old host are attacked no more or very lightly" (Steiner).

Among free-living Nematodes and also those parasitic on animals, while there are no cases so well authenticated as those quoted above, it seems more than likely that the same phenomenon will eventually be shown to occur. Steiner, for instance, records initial difficulty in transferring a polyphagous species of *Cephalobus sublongatus* in culture from one medium to another, and similar difficulties were encountered with species of *Rhabditis* and *Diplogaster*. Other cases can be cited from the work of Potts (1910) and Johnson (1913).

Among Nematodes parasitic on animals there is, as far as I have been able to discover, no completely satisfactory case, although it appears highly probable that further work will reveal many instances (see Sandground, 1925, 1929). The *Ascaris*, found in man and the pig, are often regarded as biological races of one species, on the ground of the negative results of reciprocal cross-infection experiments, and have even been given sub-specific names (*A. lumbricoides hominis*, *A. l. suis*, Martin, 1926), but I am indebted to Dr H. A. Baylis for pointing out to me the great difficulties that are often encountered in infesting animals experimentally, even with their own strain. These facts and the phenomena of age resistance introduce so many "unknowns" that, at present, the experiments can hardly be regarded as conclusive.

BIOLOGICAL RACES PRODUCED EXPERIMENTALLY.

One may turn now to a series of rather different cases, theoretically even more interesting, in which biological races have been produced directly by experiment. Some striking instances of this have already been described in dealing with the Nematodes; and other cases in which a new race appears to have been produced in a relatively short space of time by the agricultural or other activities of man (e.g. *Cerambycidae*, *Rhagoletis*, *Phylloxera*, *Chrysomphalus*) have been mentioned above. Some of the cases which must now be considered need not be recounted in very great detail. Owing to the close bearing they have on the thorny and perennial problem of the inheritance of acquired characters they are already widely known.

The earliest experiments on this line were those of Schroder (1903), who transferred the larvae of a Chrysomelid beetle, *Phratora vitellinae*, which mine in the smooth leaves of *Salix fragilis* to the downy leaves of *S. viminalis*. The larvae at first had great difficulty in mining in the downy leaves, but during four generations they gradually developed the power to excavate the downy leaves, and in this period the percentage of adults choosing *S. viminalis* for egg-laying rose from 9 to 42 per cent. In fact, a new biological race adapted to life on *S. viminalis* was being produced.

In 1908 P. Marchal was led to the conclusion that a species of scale insect, *Lecanium robiniarum*, found on a robinia introduced from N. America, had not been introduced with it but had arisen independently in France. He therefore took the closely allied *L. corni* from peach trees and transferred young scale to the locust. These matured as the typical locust form *L. robiniarum*, and Marchal found that it was exceedingly difficult to restore the offspring of these insects to the original host. Unfortunately, owing to lack of facilities in Paris, where Marchal was working, the work was not continued further, and the experiments were not performed on a sufficiently large scale to enable any important conclusions to be based upon them.

Later, in 1911, Pictet, experimenting with larvae of *Lasiocampa quercus*, produced a pine-feeding race by transferring the young larvae to pine needles. At first there was a heavy mortality, as the jaws of the larvae are constructed for feeding on flat leaves and do not open sufficiently widely to enable them to encompass the needle at the base. Only when they had learned the trick of commencing to feed at the tip of the needle (a habit foreign to them as they normally commence feeding at the base of the oak leaves) were they able to establish themselves on the pine. When once established, however, a pine-living race was formed, and even the second generation showed distaste for the original food and could hardly be induced to return to it.

Harrison and Garrett's work on induced melanism in Lepidoptera needs no recounting and does not concern us immediately, as the dark forms characteristic of industrial areas cannot of course by any stretch of imagination be described as biological races. Harrison's next (1927) paper is of greater interest, for it deals not with a direct effect of unusual environment upon the germ cells but with a true somatic response. He experimented with a common Sawfly, *Pontania salicis*, the larvae of which are gall makers on leaves of willows. He first demonstrated conclusively that there are naturally occurring races of this species confined to different species of willows, and that the egg-laying preferences of these races are characteristic.

In other experiments, carried out over a period of six years, he was able to produce a new biological race adapted to *Salix rubra* from a pre-existing *S. andersoniana* race. He considers that his experiments indicate that an acquired character, in this case a modified egg-laying instinct, is inherited. The method adopted was as follows:

A race of *Pontania salicis* attached to *Salix andersoniana* was transferred to an isolated patch of ground, where *S. rubra* was the only willow available, the plants there being quite free from any gall-making insects. The female sawflies began to lay eggs on this form, and although at first, as in the other cases quoted, the mortality was very high, some survived, and in four years' time there was a flourishing colony. Specimens of *S. andersoniana* were then planted in among the other willows, but during the three years in which observations were made none of the sawflies showed any disposition to return to this plant. Harrison concludes that in other words the "acquired habit of oviposition" on the new species had become germinally fixed

and the sawfly race was "being forced along an evolutionary path away from the parent species."

These experiments are of very great interest, and are perhaps the clearest and most satisfactory case known among insects, of the artificial production of a biological race. However, the interpretation put upon these results by Harrison in stating that the new habit was germinally fixed, seems open to question. He does not seem to have considered the possibility that his results may be due to some sort of "larval memory," the adult having a tendency to seek out for oviposition a plant of the kind upon which she had fed as a larva. While perhaps unlikely, such an explanation is by no means impossible, and has been invoked before now to explain such cases as the provisioning instincts of the hunting wasps. Indeed, such a theory is suggested by the very words of Walsh's "host selection principle," in its original form. If such host plant changes *are* of any significance as initiating evolutionary divergence, the postulation of a "mnemonic" preference keeping the nascent race to its new host, perhaps for a long period, till germinal differences appear, seems difficult to dispense with. It would also be of great interest to know if mating preferences were developed along with the change in oviposition response. As we have seen, they are known to occur in natural biological races, but it is hardly conceivable that these could be *immediately* developed as a direct result of food plant change. If, however, a preference for a particular plant was handed on to the adults from the larvae the individuals attached to one plant would, one might suppose, be more likely to mate together than with members of another host race merely because of their proximity; and it is not difficult to imagine how a mating preference might then in time be built up as an indirect result of the food plant change.

Tillyard's suggestion that a more vigorous race of *Aphelinus mali* has been produced in New Zealand by hybridising three strains from different areas does not appear to have been substantiated (Muir, 1928). If the New Zealand race is indeed more vigorous, it is more likely to be due to the direct effect of climate rather than the result of hybridisation.

Finally there are one or two anomalous cases which, although as yet little understood, are of quite unusual interest. Previous to 1916 it had been noticed by Quayle and others that in the Corona district of Southern California it was becoming increasingly difficult to kill Red Scale (*Chrysomphalus aurantii*), an introduced pest of Citrus, by the usual means of fumigation with HCN. At first this was put down to some defect in the fumigation procedure, but it soon became evident that this was not a satisfactory explanation, for by 1921 other areas in Southern California were involved, and the trouble appeared to be spreading gradually outwards from the centres where it was first observed. That the effect was due to the presence of a resistant form of the scale itself, and not to the effects of local climatic conditions on the efficacy of the fumigation process, seems to have been shown conclusively by the work of Quayle (1922). He took large samples of scale from resistant and non-resistant localities and fumigated them simultaneously under identical conditions in a third locality, with the result that the percentage of survivors in a population from a resistant area was in every case far greater (10 to 20

times) than was that from a non-resistant locality. Since that time the resistant area has steadily increased, and by 1928 included a considerable portion of the adjacent counties of Riverside, Orange and Los Angeles. In these areas the dose of HCN now required to give satisfactory results is so large as to be injurious to the trees. A similar reaction is exhibited by the Black Scale (*Saissetia oleae*), which first showed signs of developing resistance in 1912, the resistant form now having spread through a large area of Los Angeles and San Bernardino counties. The San José Scale (*Aspidiotus perniciosus*), Melander (1914), shows a similar tendency in resistance to lime-sulphur sprays, while the strains of Codling Moth (*Carpocapsa pomonella*) from widely separated regions show, according to Hough (1928), a very striking difference in susceptibility to arsenical sprays, the hybrids being intermediate in this respect.

The obvious explanation is of course that continued fumigation results in the artificial selection of more vigorous individuals, thus in due course building up a vigorous and resistant race. But this fails entirely to explain the situation in regard to the Red Scale. In this insect the resistant form crops up in a few isolated areas; from which it spreads gradually. Elsewhere, even though fumigation has been practised for as long a period, no resistant races are developed. A Lamarckian theory does not necessarily explain the facts any better. There is the possibility that the scale population of California consists of different strains originally imported from various parts of the world, and that only where a certain vigorous geographical race was originally introduced does resistance appear; but if this is so, it is hard to understand why the phenomenon was not observed from the first. Finally, there is the possibility that it might be a case of mutation induced by fumigation, only under certain restricted climatic and environmental conditions.

A great deal of work will have to be carried out before it is possible to say which theory, if any, is the correct one. It should, however, be noted that a preliminary attempt by Boyce (1928) to produce resistant races of *Drosophila* and *Aphis* by fumigation was unsuccessful. While individuals were found to vary in their vigour so that fumigation appeared to produce a slight increase in resistance, yet work carried on over seven generations failed to give definite evidence of any cumulative effect.

DISCUSSION AND SUMMARY.

Turning now to a consideration of the general significance of these numerous examples, the question which first presents itself is: Do they really represent the commencement of evolutionary change? In other words: Are they species in the making? A large number of workers (e.g. Schroder, Pictet, Cuénot, Nuttall, Harrison, Adkin and Sandground, to mention only a few) have answered in the affirmative with regard to the particular case with which each was concerned¹. It seems worth while now to consider the question as a whole. If these races are

¹ This apparently is also the point of view taken by Hering (1926 a) with regard to the Lepidoptera when he states that the polyphagous forms are phylogenetically the oldest and that monophagy is a more recent development. For a detailed discussion of the same subject, with special reference to leaf-mining larvae, see Hering, *Die Ökologie der blattminierenden Insektenlarven*, 1926, pp. 120-44. A similar view is taken by Howard (1924 b, p. 37) with regard to the parasitic Hymenoptera.

indeed nascent species, then we must assume that their characteristics are sooner or later germinally fixed and that there is some degree of isolation, physiological or habitual, which ensures their continuance. How much evidence is there for these assumptions?

It seems to the writer that perhaps the most important consideration in this connection is that of isolation. As has long been realised, the great difficulty in regarding mutations or any other inheritable variations produced in small numbers, as the starting point of new species, is that of accounting for the spread of such throughout a population. If the differences which serve to distinguish one species from another were of a kind likely to be of immediate survival value, then the problem would be simpler, but this does not appear to be the case with the general run of specific characters, still less of varietal ones. The importance of geographical isolation in fostering such variations is undoubted, but in many cases it is unlikely that this factor can come into play until a comparatively late stage in the process; the first steps in the spread of a new variant are little assisted by geographical barriers. If, however, there is some form of physiological isolation such as is provided by various small biological differences and by a repugnance for cross-mating, then the process seems much easier to comprehend. Charles Darwin saw this clearly, as is evinced by his great interest in Bates' statement (*Trans. Linn. Soc.* 23, 501): "The process of the creation of a new species I believe to be accelerated in the *Ithomiae* and allied genera by the strong tendency of insects, when pairing, to select none but their exact counterparts." As Carpenter (1913, *Proc. Ent. Soc.* p. lxxxviii) points out, Darwin (*Life and Letters*, 2, 392) wrote to Bates regarding this generalisation: "I look upon this fact as very important"; but later rather severely criticised him for making the statement in general terms without sufficient evidence. That the evidence even now is far from being as extensive as could be wished is made clear by Richards (1927, pp. 345-347).

As has already been seen in this review, in the few instances among naturally occurring biological races in which the matter of selective mating has been put to the test, the results have been positive, although not always, it must be said, on a scale large enough to be very convincing. There is here a big field for further work of a very great importance, but in the meantime it is not very difficult to conceive how a biological race may be perpetuated. Even though the mating preference might not be very strong, yet under natural conditions in combination with slight differences in habitat and time of appearance, it might stop interbreeding almost completely. Moreover, as was pointed out above, if the oviposition reaction is in some degree determined by a "memory" of the larval food, as postulated by Walsh and others, then the early stages of such divergence are correspondingly easier to understand, for we are relieved of the necessity of imagining an immediate germinal change corresponding to the new habits. A new host plant race might thus be separated mnemonically for a very long time without any germinal change taking place.

Another point in favour of the view that such races are of some evolutionary significance has been brought out by Richards (1927) in a different connection. He

says: "If the first barrier in the divergence of species were a physiological one of this nature it would be easy to see why the sterility of interspecific crosses should be so capricious in its incidence, for it might arise more or less accidentally, at any time after the species had ceased to interbreed."

These considerations, then, indicate how a new race might be developed apparently irrespective of the action of natural selection, provided of course that the variation was not positively harmful. In the case, however, of a plant-feeding insect the production of a new host form might be of very great value in that it might enable the insect to occupy an otherwise vacant ecological niche previously closed to it, and thus to colonise a large area. That the correlation of such important biological differences with minute morphological characteristics of no survival value may supply an explanation of the persistence of these latter has of course long been realised.

It also seems worth considering what bearing these facts may have on the rather academic question as to which comes first—physiological or morphological variation—academic because of the difficulty or impossibility of drawing any line of division between the two. Robson (1928, p. 183), discussing Darwin's view that it is immaterial which comes first or whether they both come together, says: "I do not think we can regard the matter as immaterial until we know more about the actual origin of habits. The doctrine of Natural Selection and Lamarckian argument do not require as an absolute condition that the acquisition of a new habit should precede a structural change; though, speaking broadly, both theories would be easier of acceptance if this condition were fulfilled."

It will be obvious that the various cases described above do supply some very striking instances of significant physiological change, either without any visible morphological variation or with the latter relatively very slight. When morphological change is present it is, it is true, often extremely difficult or impossible to decide which came first, but we can at least say that, whereas the physiological changes are important and likely to be of survival value, the morphological ones are trivial in the extreme.

It seems then, that although much more evidence is desirable, there is no very great difficulty in visualising how a biological race having once begun, may persist and increase. The next question is—if this is a mode of evolution, how consistently does it operate in nature? Meyrick (1927), for instance, tends to restrict the application of the principle on the ground that many closely allied species have the same food.

In this connection let us consider first the phenomenon of large-scale habit change which is such a feature of modern economic entomology, and which, as is well known, occurs in many groups of animals (see Ritchie, 1920, pp. 400–416). There are many cases on record of a large scale and relatively sudden change of habit (*e.g.* food, oviposition preference), as a result of which a species is able to colonise new areas or new environmental niches. Such new niches may then be occupied apparently in preference to, if not to the exclusion of, the old. Robson (1928, pp. 110 and 156) lists several interesting cases of this in groups other than

insects. Among insects the theory of Anopheline zootrophy and the cases of *Rhagoletis pomonella* have already been mentioned, and such cases as the Colorado Beetle, *Leptinotarsa*, and the Parsley Stalk-Weevil (*Listronotus*), Boyce (1927), come to mind. A particularly interesting case, in that the whole process has been observed, is that of certain Capsid bugs in the fruit-growing areas of East Anglia. *Lygus pabulinus* has long been known to Hemipterists as one of the commonest Capsids to be obtained in summer by sweeping herbaceous plants; it was not known previously to 1928 that it was bi-voltine, the winter eggs being laid in the stems of woody plants. From the work of Petherbridge and Husain (1917) on the Capsid pests of apple in East Anglia, it is certain that *L. pabulinus* did not at that time occur on apple, and was rare on currants or other bush fruit. By 1926 it was found frequently on apple and had become a serious pest in currant and gooseberry plantations, owing to its newly acquired habit of using these plants for a winter host. In this area it appears to have deserted the original winter host, apparently blackberry, almost entirely (Petherbridge and Thorpe, 1928).

Although it is not proved in any one case, with the doubtful exception of *Rhagoletis*, that such habit change is correlated with the development of a separate biological race; yet in view of the rapidity with which, in certain instances, such races appear to have been produced experimentally, it seems more than likely that this is the case.

So much for the evidence of the plasticity of food habits; but viewing the matter from another standpoint it at once becomes obvious that such rapid development of new races cannot be going on uniformly in nature, or there would be no specific stability at all. In reality there are many instances in which it seems certain that the feeding habits of a species or genus of insects must have remained constant over a very long period of time. Such cases are postulated by Harrison's theories (1920, 1924) as to the origin of the different races and species of *Aricia* and *Oporobia*, and Brues (1920) cites the instances of the long period during which the holarctic and nearctic Vanessids or the world-wide *Aristolochia*-feeding Papilios must have remained constant in food habits. Constancy of food habits might, of course, be explained on the assumption that great mortality is always caused at the commencement of a host plant change, and that under natural conditions the few survivors would have an extremely slender chance of perpetuating the newly formed habit. Nevertheless, were the food habits of all insects as easily modified as, say, those of *Pontania* or *Rhagoletis* appear to be, even such initial difficulties at establishment would not suffice to account for the case of the Papilios and Vanessids cited above, nor for many others.

It will have been realised from what has been said already that there are certain difficulties in the way of considering the host selection principle as of wide application in the production of new forms, especially in that the stronger the tendency of a species to develop a host preference the more difficult it becomes for that species to adopt new hosts. If, however, as seems possible in many cases, the host preference is mainly upon the psychological plane, without any *physiological* inability to change to a new host, we may have an explanation of certain of these

difficulties. Thus, under normal conditions, the insect would remain constant, but during periods of exceptional stress, due perhaps to climatic or other environmental changes, the psychological preference might be overcome, with the result that new host plants would be adopted and a new race thus developed.

We can only suppose in our ignorance that while fixity of habits is the rule, species and genera pass through periods of plasticity, perhaps coinciding with particular climatic or environmental changes, during which habits are easily changed and during which the origin of new races and species proceeds apace, to be succeeded perhaps by another long period of staid conservatism.

Finally, since certain workers in this field have regarded their results as evidence of the inheritance of acquired characters, the question presents itself: Can the origin of biological races be explained on any non-Lamarckian theory? And this in turn depends on the question: Is the evidence really satisfactory that selection of pre-existing strains will not explain the phenomena?

In the majority of experiments quoted above, there is certainly no *proof* that the results could not be explained on the basis of selection from a mixed population; this is especially the case in experiments such as those of Pictet (1911) on *Lasiocampa quercus* and Schroder (1903) on *Phratora vitellinae*, in which there was a very great initial mortality. It must be said, however, that such an explanation seems highly inadequate for most of the results described, and in one or two cases it appears to be ruled out almost certainly. Thus, whilst a number of the Nematode experiments could be accounted for in this way, the theory seems quite inadequate to account for those instances in which the Nematode population becomes so specialised on a new host as to be unable to return to the original food plant. Another difficulty is found in the work of Tischler (quoted above), in which it is stated that, if left to choose, the *entire* population goes to *Circaeа lutetiana* and *none* to *C. intermedia*, but that if the former is absent infestation of the latter will take place. It seems that such a result, if adequately substantiated, cannot be explained on any theory of selection. Unfortunately the subsequent behaviour of a strain forced on to *intermedia* does not appear to have been studied. In view, however, of the long period during which some Nematodes are capable of living without nutriment and of our uncertainty as to the factors which govern ability to infest fresh hosts, it is perhaps unwise as yet to lay too much stress on these points. It should also be mentioned that, because of the resistant eggs and long life of the adult resulting in the probability at any given time, that all the various stages of the Nematode will be present, it is far more difficult to eliminate the possibility of a "mnemonic" theory than it is in experiments with insects. Such a theory, however, seems quite as incapable as that of selection of accounting for the extreme cases just cited.

There are also serious difficulties in the interpretation of some of the insect experiments on these grounds. Thus Harrison (1927) states that of the experimentally produced *Salix rubra* race of *Pontania*, *none* returned to *S. andersoniana* in the course of three years. Since, as was remarked above, the possibility of "memory" was not eliminated this case cannot be regarded as conclusive evidence; a possible explanation of the results might be as follows. Suppose the Sawflies on

the initial food plant *X* to be composed of two strains: *A*, capable of development on both plants *X* and *Y*, and *B*, capable of development on *X* only. Suppose also that both these have the "mnemonic" preference for oviposition on *X*. They can thus be represented as *Ax* and *Bx*. Now force this population on to host *Y*. By selection *B* is then eliminated, but strain *A* establishes itself, and in the second generation its mnemonic preference becomes changed so that we can represent it as *Ay*. If this population *Ay* is now given a choice of *X* and *Y*, it will remain on *Y* but could easily be *forced* back on to *X*. Only if there was *difficulty* in doing this, or if a continued preference for *Y* over *X* was shown in the second generation or after—only then should we be entitled to put forward a Lamarckian explanation.

It must be said that this explanation is perhaps unlikely. Nevertheless its possibility should be considered.

In the work of Nuttall and others on the transformation of *Pediculus capititis* into *P. corporis* we come up against the difficulty that the change did not appear suddenly in the first generation after the change in feeding methods, but developed gradually over at least four generations. Keilin and Nuttall (1919, p. 325) expressly state that Lice, *P. capititis*, which had been reared by Bacot for two years in the laboratory, were *intermediate* in structural characters between typical *capititis* and *corporis*. Here again it is very difficult to find an orthodox explanation.

In conclusion, then, we may say that many of these experiments are easily explained on some form of Lamarckian theory, but extremely difficult to account for on any other lines. If the hypothesis were not such a debatable one the evidence might well be regarded as almost conclusive for, to quote Thompson and Parker (1928), "it is probably the only intelligible theory of a natural evolutionary process ever advanced." It seems equally certain, however, that none of the experiments recorded in these pages has been on a sufficiently extensive scale to carry complete conviction on the point. They do, however, suggest most profitable fields for further work of this nature and, taken together, they provide a quite considerable amount of the ever-growing body of circumstantial evidence for the theory.

APPENDIX.

- (1) See also PAINTER, R. H. (1930), "The Biological Strains of the Hessian Fly," *J. Econ. Entom.* 23, 322–6.
- (2) A. PETERSON (*J. New York Ent. Soc.* 38, 1930) also brings forward evidence which points strongly to the existence of two distinct biological races in N. America.
- (3) See also ROUBAUD (1929), *International Corn Borer Investigation Sci. Reps.* 2, 1–21.
- (4) A. HASE (1929), *Int. Corn Bor. Invest. Sci. Reps.* 2, 85–89, also reports that no special host preference was developed with *Trichogramma evanescens* after breeding on *Ephestia* and *Galleria* for three years.

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THE PHYSIOLOGY OF HIBERNATION

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(Received February 17, 1930.)

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I. GENERAL.

IT is a property of living organisms to withdraw from an environment, when it becomes too unfavourable. This withdrawal may be effected by migration, but should this be impossible, the metabolic processes of the organism are reduced to a minimum, and it remains torpid till more favourable conditions obtain once again. This property is a fundamental one, and in the lowest organisms is often associated with reproductive activities.

As it happens, in the countries where the bulk of physiological research is performed, this withdrawal takes place in winter. Moreover the physiologists are chiefly interested in the mammalia, the result being that too much attention has been paid to the special case of mammalian hibernation, instead of to the more general problem outlined above. (See, however, Carlier, 1911; Pocock, 1926.) The conflicting ideas resulting from this narrow point of view drove Horvath to state that the winter sleep is not a sleep, and has nothing to do with the winter.

Pocock (*loc. cit.*) has given a very good account of the general zoology of hibernation, therefore only cases of special interest will be mentioned here. In general, all poikilotherms which cannot emigrate from regions that have a severe winter hibernate. Thus, marine fishes do not hibernate, whilst certain fresh-water fishes do so (Knaute, 1894, 1896), and many fixed shallow-water organisms in the Mediterranean aestivate.

At first sight, it appears that the problem of poikilothermic hibernation is quite simply defined: the cooling of such animals must result in a slowing of their activities, and the puzzle to be unravelled is to determine the physiological changes

that enable the tissues of adapted organisms to recover from such a cooling, and why this is impossible in an unadapted animal. It also remains to be determined how it is that certain cold-blooded animals are active at temperatures at which nearly related species are dormant. Thus *Planorbis* may be seen creeping about under the ice. Drewson (1845), working at Heidelberg, stated that *Olophrum piceum* and *Acidota crenata* are active at 8° C., and Smith (1902) records cases of mosquitoes that do not hibernate. It may be argued that the activity of *Planorbis* is merely a special case of the adaptation that is mentioned above, but evidence will be brought forward to show that not only must a hibernating animal adapt itself to cold, but also that it must adapt itself to its winter quarters in other ways. As far as the insects are concerned, the observations of Marie Parhon (1909) on bees suggest that they may have, at any rate, the rudiments of homiothermy. Also the problem of aestivation of cold-blooded animals (as for example of the lung fishes *Protopterus* and *Lepidosiren*) remains to be considered. Although in this case there can be no question of a passive cooling, nevertheless there is evidence that suggests that the adaptive changes are not dissimilar to those occurring in hibernation.

In the case of warm-blooded animals one wants to know not only how their tissues adapt themselves to low temperatures, but also to what somatic and environmental changes their dormancy and temporary loss of homiothermy are due. Actually it is with the latter problem that nearly all the theories of hibernation deal.

As the possible environmental factors are far less numerous than the organismal factors, we will consider the former first. As Pocock says, the mere fact of aestivation should dismiss cold as the prime factor in initiating dormancy. Moreover, Valentin and Horvath (1881), Quincke (1882) and others have noticed marmots to become dormant in summer. Similarly, Forel (1887) records the case of some dormice that remained awake all winter and became dormant in the heat of the summer. In fact, most workers have noticed how liable a hibernating mammal is to lapse into torpor and become poikilothermic. Lastly, as Mangili (1808) points out, very severe cold causes waking. The next factor to receive consideration is the food supply. Simpson (1911) has dealt with this at some length, and though it appears that this factor is of greater importance than the temperature, the results are very conflicting. It could easily be objected that it is doubtful how much importance can be attached to results obtained under laboratory conditions, but as Cleghorn (1910) points out the marmots of the lower Adirondacks normally retire in September when the weather is warm and food is plentiful. The next constituent of the environment to be considered is the atmosphere; Bert (1868) considered the predisposing factor for the initiation of winter sleep was deprivation of oxygen followed by an increase in carbon dioxide concentration. However, this theory does not explain why animals which normally do not live in a confined atmosphere should seek one. Altogether the facts make it evident that the environment alone will not afford a satisfactory reason for the assumption of torpidity. There is obviously a great individual difference amongst members of the same species in this regard. Thus Rasmussen (1916 c) remarks that amongst the colony of marmots he had under observation, some would be in the most profound

torpor whilst others would be fully active. Similar observations have been made by Prof. Pembrey and the author. Between different species the differences are naturally greater. The liability of dormice to go to sleep at high temperatures has already been noted (see also Berthold, 1837, who records dormancy occurring at 16° C.). On the other hand, Mills (1892) states that bats can be made dormant or active at will by varying the temperature. By way of contrast, the behaviour of the Madagascan tenrec (*Centetes ecaudatus*, an animal closely related to the hedgehog) may be noted. Pocock noticed that it became dormant regularly at the same time every year in the Zoological Gardens, although kept under uniform environmental conditions. This animal is usually stated to aestivate at the hottest season of the year, although this has been disputed (see Pembrey, 1895).

Thus, although undoubtedly in a general way it is true that the withdrawal from the environment is a withdrawal from unfavourable conditions, it is impossible to point to any one condition as being responsible, even for the same species of animal. One must therefore seek for the common factor in the physiological changes, even though these be produced by widely different environmental factors.

The number of theories of hibernation is enormous, and the reader is referred to a review of them by Rasmussen in the *American Naturalist* (1916 a).

In the light of the facts stated above, it is obvious that any theory that over-stresses the responses of the organism as a response to cold cannot have a universal significance. Therefore statements, such as that of Claude Bernard, that the cause may be found in a special susceptibility of the nervous system to cold must be dismissed. As was stressed at the beginning of this article, the withdrawal from the environment is a very primitive attribute: even in the case of man it is never entirely lost. Luciani (1921) mentions the case of Anna Garbero, who remained in a trance for over two years without nourishment. This case, like that of the fakir's trances, is looked at askance, although the latter are vouched for by such an authority as Verworn (1915). However, in Russia, in the region of Pskov, the peasants spend most of the winter asleep round a fire, waking but once a day to eat a very little bread (Volkoff, 1900). All animals when asleep tend to show a drop in temperature. The primitive mammalia as shown by Martin (1901), and the young of the more advanced types (Pembrey and Hale White, 1896), are specially liable not to maintain a constant temperature. Thus Edwards (1882) and Marshall Hall (1838) compared hibernation to sleep. As early as 1778 Pallas drew a comparison between a foetal mammal and a hibernating mammal, whilst Pembrey (1895) and Merzbacher (1904) have stressed the likelihood of a primitive organisation of the nervous system in such animals. Even now, there is no unanimity in describing sleep in terms of physiology. If Pavlov's explanation of a widespread inhibition is accepted, a large number of apparent differences can be explained on the ground that the distribution of the inhibition throughout the nervous system differs from animal to animal. Very recently Bruman (1929) has stressed the importance of the parasympathetic in normal sleep and in hibernation. He points out that both atropine and adrenaline cause waking. It should be pointed out, however, that although the parasympathetic

undoubtedly tends to inhibit those activities that are associated with muscular activity, it tends to excite those activities associated with ingestion and excretion in the alimentary canal, which are at a minimum during hibernation; indeed the presence of faeces, etc., tends to cause waking. Both hibernation and sleep are not unlike anaesthesia, and accordingly Dubois (1896) has brought forward the theory that a dormant animal is in a state of carbon dioxide narcosis. He has some fairly good evidence to support this view. For instance torpidity can be induced by exposing the animal to air containing 45 per cent. carbon dioxide; higher concentrations cause a dormant animal to wake up. He also points out that the carbon dioxide concentration of the blood is raised during dormancy. However, Rasmussen (1916 *b, c*) has shown that although it is sometimes raised to as much as 100 vols. per cent., it may be no higher than that of an active animal. It may be noted that, whereas Verworn (1915) says that fakirs breathe as little as possible before entering a trance, other people say just the reverse (Buchanan, 1923). Indeed Mosso (1899) has postulated acapnia as the cause, and Buchanan (1923) succeeded in inducing a state resembling hibernation by means of over-ventilation and exposure to cold. Now, in view of Dubois' and Rasmussen's results, it is obvious that this theory is open to criticism. In short, the accuracy of temperature control of any animal is very liable to diminish when asleep, under anaesthesia or after over-ventilating. Merzbacher (1904), Simpson (1911) and numerous others have noticed that the temperature of an animal that hibernates is specially liable to vary, and under the conditions outlined above is almost certainly more prone to lose control of its temperature. In any case, none of these theories deals with what is really the more fundamental problem, namely, how such an animal is able to tolerate such a large range of temperature, and further how the tissues of an organism can remain unharmed for long periods, even at a high temperature, when completely deprived of nourishment.

Till now we have been dealing almost exclusively with the peculiarities of the nervous systems of hibernating mammals, and it will be as well to consider what other somatic changes may occur as a prelude to, or during, hibernation. Sacc (1858) was the first to suggest that the fatness of an animal was one of the prime predisposing factors. Valentin and Horvath (1881) recorded that the marmots which became lethargic in summer were very fat. Cushing and Goetch (1915) have described fat and lethargic animals produced experimentally. Such an animal is a young animal that has had its pituitary removed. It is worthy of note, in light of the observations of Pembrey (1893, 1894, 1895), that the animals upon which these observations were made were young. Cushing's hypophysectomised animals show an even closer resemblance to a hibernating animal than is suggested by the fact that they are fat and lethargic; their hearts when excised continued to beat for some hours like that of a batrachian, or like that of a hibernating animal (see also Cushing's article in the *Lancet*, October 31st, 1925). Gemelli (1906) records that the pituitary of a hibernating marmot shows a great decrease in the chromophile cells, whilst on waking there are to be found a large number of karyokinetic figures. These findings were confirmed by Cushing and Goetch. Unfortunately, as in the

case of every change mentioned, these phenomena have been shown not to be constant: Mann in 1916 was able to confirm that they did occur, but he also showed that an animal may hibernate quite normally without their occurrence.

Although these changes must be of significance, there is apparently some far more fundamental change that must be looked for before any generalisation can be made. It must be remembered that the pituitary, important though it is, acts by modifying the *milieu interne* of the organism, and it is to changes of the internal environment that we must look for a solution of the problem.

In my opinion it is not in any work on mammals published up to date that this clue is to be found, but rather in the work of Tower (1906) and Breitenbecher (1918) who studied the hibernation of the potato beetle. Tower thought at first that cold was the prime cause, but later he came to the conclusion that this was not so, and that various strains differed from one another in their behaviour in respect of hibernation. For some strains it appeared that food was the prime factor, in others moisture, etc. Finally Breitenbecher showed experimentally that drying was of prime importance, even in the presence of ample food supply. Tower had noticed previously that these beetles dehydrated themselves when hibernating under normal conditions. These animals could be kept dormant for considerable periods at a high temperature by being kept in a dry atmosphere (although it should be mentioned that the death rate was fairly high).

To sum up: *Various animals when confronted by an unfavourable environment undergo physiological modifications which result in torpidity. The identity of the most important component of the environment depends on the phenotype and the species. Torpidity will result if these changes are brought about by other than the normal stimuli.*

II. THE METABOLIC PROCESSES DURING LETHARGY.

(a) Respiratory exchange.

The next step is to determine as far as possible what the physiological modifications are that accompany torpidity.

The evidence on this point has been gleaned almost entirely from a study of the respiratory exchange. A tremendous controversy has centred round the value of the different results obtained. Unfortunately (as far as I am aware) no quantitative results have been obtained from animals lethargic at high temperatures. Such data would go a very long way to decide how far results obtained from hibernating animals are vitiated by the effect of temperature. The literature on the respiratory exchange is very large, and concerns itself chiefly with the question of how far the low respiratory quotients that have been observed are artefacts, and how far they are to be regarded as valid indices of metabolic activity. A paper dealing with this subject in detail is in preparation by Pembrey and Gorer.

In spite of the differences that occupy the literature two generalisations may be made. (1) That the metabolic processes of a lethargic mammal are faster than those of a cold-blooded animal under similar physical conditions (Athanasius, 1909). (2) That both groups of animals tend to obey the surface law

even when torpid (Maurel, 1900; Athanasiu, 1909; Pembrey, 1895). These values for the metabolic rate are of importance because they are as near the minimum required to maintain the structure of protoplasm as one is likely to get. Similar values obtained during aestivation would be of even greater value in this respect, because the metabolic rate of hibernating animals follows the temperature rather readily, whilst it seems very unlikely that this can be so for organisms that remain inert for long periods at high temperatures. The highest rates for a hibernating mammal are those of the dormouse (Pembrey, 1903). Awake, it was about 8000 c.c. O₂ per kg. per hr.; asleep at 10° C., about 300 c.c. The marmot uses about 550 and 35, awake and at 10° respectively. Athanasiu (1900) gives figures for the frog and Regnault and Reiset (1849) give figures for the lizard. Athanasiu gets the surprising result of a quotient of unity for the frog. A full list of results on poikilotherms is given by Pembrey (1895).

As far as the quotients are concerned physiologists may be divided into two camps, those who believe in the validity of low quotients and those who do not. It may be said at once that the majority of observers working on animals of various kinds at low temperatures have obtained low quotients. (See Krogh, 1916; Battelli and Stern, 1913.) For hibernating mammals Valentin, Voit (1878), Regnault and Reiset (1849), and Pembrey (1901, 1903) have obtained quotients which vary between 0·6 and 0·3 on the average, but have even sunk as low as 0·25 (Pembrey, 1901). There have been numerous objections to these findings, but on the whole it must be admitted that certain technical improvements such as tracheotomy (as performed by Henriques, 1911) are very likely to waken the animal. If care is not taken a profoundly lethargic animal may be wakened during transference to a respiration chamber. More important objections are those that stress the vitiating effects of the enhanced solubility of carbon dioxide at low temperatures. Pembrey and Gorer (1929) have performed experiments over long periods ranging up to 48 hours so as to enable equilibrium to be attained, and the conclusion reached was that the carbon dioxide retention required to account for the low quotients was an impossibility. Further, Hari (1909) who, like Nagai (1909), objects to the low quotients, found a value of about 0·5 over a period of 26 days.

Further evidence against accepting the explanation of carbon dioxide retention may be obtained from the quotients during waking, which often do not rise above 0·7 (Pembrey and Gorer, 1929; Marès, 1892). It should be mentioned, however, that Dubois (1896) and Wienland and Riehl and others have observed quotients of 1·0 or over. Such discrepancies, however, do not necessarily indicate an error in technique, the former history of the animal as regards diet, reserves of fat, etc., as well as individual variations, would account for differences in metabolic processes during waking. Accepting the lower values, it is obvious that if much carbon dioxide is being blown off whilst the metabolism and temperature are rising, the real quotient must still be below 0·7.

A quotient below 0·7 means in general terms a partial oxidation, probably of fat. That this may take place is suggested by analogy to results obtained on starving animals, and Valentin (1857) finds an acid urine during hibernation of herbivores

and Rasmussen (1916 *b*) a decreased alkali reserve as measured by the carbon dioxide absorptive power of the blood. In other words there is a certain amount of evidence that there is a tendency to acidosis brought about by the acids produced by the partial combustion of fats. Nagai (1909) obtained further proof of the partial oxidation alluded to above. He found from 25 to 120 mgm. of lactic acid per kg. per day excreted in the urine, also that the nitrogen metabolism is decreased to a far smaller extent than that of other substances: the nitrogen excretion is reduced to one-sixth of the non-hibernating value, whilst the oxygen absorption is diminished 20 times. How far the partial oxidations which these results prove to occur are quantitatively sufficient to explain the low quotient Nagai did not show.

Another possible result of such an oxidation is the desaturation of fats, which is known to tend to occur at low temperatures (Leathes, 1925). The last possibility is the formation of glycogen. The question of its derivation from fat is one of the most fiercely contended in biochemistry. That it is chemically possible can hardly be doubted in light of Ivanov's (1912 *a* and *b*) experiments on the germination of seeds. Bernard, Voit, Aeby (1874), and Wienland and Riehl (1908) have found a high glycogen content in the marmot, but their values cannot be justifiably held to indicate an increase during hibernation as some have done. Athanasiu (1899) and Pflüger (1898) have found as much as 1·0 per cent. of glycogen in the frog towards the end of hibernation. This figure appears enormous, but it must be remembered that determinations on glycogen content have not generally been made on hibernating animals. Krogh objects, in the first place, that these low quotients are unreliable, and secondly that if they were correct an incredible amount of glycogen would have to be formed during a long period of dormancy. This latter objection applies to any other product of partial combustion that may be selected. As to the former, it is the author's opinion that the metabolism of a torpid animal is truly represented by quotients between 0·3–0·5, say on the average 0·4. In short, one must reach the unsatisfactory conclusion that there is no more to be gained by discussing the respiratory quotient, and that what is wanted is direct evidence on the metabolism, which should settle the question of the quotient far more readily than the quotient can decide on the type of metabolism.

If this question of metabolism could be decided, the next question would be how far the phenomena observed are due to a low temperature, and how far they are characteristic of torpidity. Here experiments relating to animals dormant at relatively high temperatures should settle the matter, and would throw invaluable light on the fundamental mechanism of the withdrawal from the environment. One would like to know, moreover, what are the factors that limit the range of temperature to which an animal is tolerant.

It seems probable that a large part may be determined architecturally as, for instance, by a modification of the lipoids as suggested by Tait (1922). But that there may be qualitative metabolic changes as well is indicated by Braem's (1890) observation that the statoblasts of certain fresh-water polyzoa will not develop unless submitted to a temperature in the region of 0° C.; to ensure that this will be obtained the statoblasts are provided with floats.

There can be little doubt that the temperature at which an organism remains for any length of time modifies the responses of its tissues to a change of temperature, as is shown by Hogben's (1925) observations on the contractile tissues of marine invertebrates. Montuori (1907) indeed found that the metabolism of fishes acclimatised to temperatures above 25° was less than it had been at about 11°. As Krogh quite rightly says, these results cannot be accepted without reserve, and it is well known that fishes in an aquarium at these high temperatures are sick animals. However, Bodine (1923) found that the metabolism of a grasshopper that had been hibernating was considerably higher after an hour at room temperature than that of controls that had been in the laboratory for some days. This result agrees well with those of Hogben, but it is only fair to state that Bodine draws a very different conclusion from these findings. Many observations have been made on the excised tissues of hibernating animals, as well as on the respiratory movements and the hearts *in situ*. Thus Valentin (1870), Dubois (1899), and Patrizi (1894) find that excised muscles of a hibernating mammal can contract for some hours after excision, but that the reactions are naturally very much slowed. Bürker (1905) found that in the case of the frog's muscle, the influence of tension on the heat developed was greater in winter than in spring, and that the rise in temperature for any given excitation was greater in the spring than in the winter. Succow (1909) measured the cardiac rate in insects during hibernation, and found it reduced by about 25 times. Valentin (1860) finds that the rate of mammalian hearts depends not only on the temperature but on the size of the animal. Buchanan (1911) has used electrical methods of recording the heart rate of the dormouse and finds there is a heart block.

The respiratory movements have been studied by many, the most valuable observations being those of Pembrey and Pitts (1903), who correlated the internal temperature with the type of respiration observed, and found that there was a transition from normal, to Cheyne-Stokes, to a Biot type as the temperature fell. The observations of Bailey (1817) that a hedgehog can withstand pure nitrogen for 5 minutes or that of Spallanzani (1895) that a hibernating mammal can withstand pure carbon dioxide for long periods, do not prove as Pocock (*loc. cit.*) claims that respiration ceases. Rasmussen (1916 b) has shown that the blood may be nearly saturated with oxygen, and there would probably be enough to tide over a considerable period. The second observation is very surprising in light of Dubois' (1896) observation that a very high carbon dioxide tension causes waking. One can only assume that narcotisation took place, and that the altered metabolism and very slow respiratory movements prevented the actual dose of carbon dioxide from becoming lethal.

These observations on the activity of tissues during hibernation are of interest, but the interest would be enhanced if comparisons were made on the effect of raising the temperature of such tissues, with similar experiments on cooled and warmed tissues of active animals. The excised muscles of an active marmot, for example, behave like those of any other animal. It would be valuable to repeat the experiments, using marmots rendered poikilothermic by anaesthesia, and com-

paring the results with those of other non-hibernating animals in the same condition. Finally, it would be of great importance to discover if the haemoglobin differed, as far as its thermal coefficient is concerned, during lethargy and wakefulness, in the same way as Macela and Seliskar (1925) have shown that the haemoglobin of a poikilotherm differs from that of a mammal.

It should be remembered that the difference between poikilothermy and homoiothermy is not sharply defined. The observations of Marie Parhon on bees have already been mentioned; even more striking are the observations of Valenciennes (1841) on the heat generated by a brooding python. In any case there can be little doubt that an indiscriminate application of various thermochemical laws does not tend to clear up the problems. For example, de Heyde (1921 b) found that the thermal coefficient of urinary secretion in a frog was positive as the temperature rose, and Martin (1901) indicates that the same is true for the primitive mammalia. It is of interest to note that de Heyde obtains evidence that there are qualitative urinary changes as the temperature varies. As a matter of fact Krogh finds that a negative coefficient is the rule, but whatever may be the more general phenomenon, there can be little doubt that adaptive changes do occur and, as has been said already, one wishes to know what these changes may be. In the case of the lower organisms it appears that adaptive changes take place by the direct influence of the external environment; as one ascends the animal scale one finds the tissues more and more shielded from a varying environment, and the internal environment being maintained more or less constant. Therefore in the case of the mammalia we must expect to find the adaptations governed, in part at any rate, by those factors which concern themselves with the constitution of the internal environment; such factors are represented largely by the endocrine system.

(b) *The endocrines.*

The endocrine system is connected most primitively with reproductive activities, and one might expect that retirement might be invariably associated with some phase in the sexual history. However, there is great variation in the state of gonads at this period. Thus some bears hibernate during pregnancy. According to Pocock, only the female polar bear hibernates and goes through labour and suckles her young for some time secluded in the ice. Mann (1916) records that the ovaries of the marmot show a rapid formation of follicles just before the winter sleep, maturation of them being delayed till the spring. In the case of an insect, however, Bodine has shown that hibernation is definitely associated with the life cycle: the grasshopper, *C. viridifasciata*, cannot hibernate after the third instar.

Before considering any of the histological changes that the various glands are said to undergo, it should be mentioned that various modifications of structure have been described for most cells in the body. Leonard (1887) has described a diminution in volume, and the appearance of eosinophile granules and fat globules in the liver of the frog; whilst Monti and Monti (1903) have described more or less similar changes in the stomach. For a fuller list the reader is referred to the review by Athanasiu (1909).

In the light of these facts, too much importance should not be attached to such changes as Peiser (1906) has described as occurring in the thyroid of the hedgehog and bat. (These changes are considerable flattening of the cells and a great diminution in colloid.) Domenico Cesa Bianchi (1907) described a complete disappearance of the interstitial gland of the ovary during the winter. As has already been mentioned, Mann insists that no histological change is constant in any endocrine; but that the most, or perhaps the only important evidence is that which concerns the pituitary. Nevertheless it should be borne in mind that lack of visible structural modifications does not necessarily exclude functional alterations. This is specially true in dealing with the endocrines, when a very slight disturbance in the metabolism of any of these glands can have the most profound effect on the body as a whole. Indeed any profound structural changes would seem unlikely, considering that an animal in the most profound torpor can, inside an hour, return to full activity under appropriate circumstances.

Another method of studying the possible importance of the endocrines has been that of injecting various extracts. Unfortunately here again the evidence is rather conflicting. The chief objections are raised by Zondek (1924), who considers that the temperature of the injected solution is of prime importance, and has wakened animals by injections of warm saline. However, Adler (1920) has wakened animals by means of thyroid extracts; and by sectioning branches of certain nerves has come to the conclusion that the effect is a direct one on the tissues. Bruman (*loc. cit.*) attaches special importance to the waking caused by injections of adrenaline and by atropine, and uses these results to support his idea of increased parasympathetic activity being largely responsible for winter sleep. This idea has been dealt with to some extent already. Further, it may be mentioned that the heart block described by Buchanan is not consistent with enhanced vagus activity. Incidentally Bruman failed to repeat Zondek's results.

As far as the suprarenal gland is concerned, Mann has described some slight structural modifications. What is more interesting is the fact that marmots survive removal of these organs remarkably well. Mann thinks this may be due to the presence of accessory suprarenals, but he is apparently not convinced, and further investigations would certainly be of interest.

Dworkin and Finney (1927) observed that injections of insulin cause marmots to become dormant, whilst injections of sugar wake them again. Apart from the interest attaching to the influence of hypoglycaemia on the nervous and other tissues, these results are significant in connection with the high glycogen values mentioned above; these fit well into the observations of Best, Dale, Hoet and Marks (1926) concerning the deposition of glycogen under the influence of insulin.

Lastly one comes to the pituitary, and one cannot fail to be struck by the analogy between the fat marmots becoming lethargic in the summer and the hypophysectomised puppies as described by Cushing. In addition, Schenk (1922) found that injections of anterior pituitary extracts cause waking. The discussion of the value of these and other injection experiments will be reserved till the general question of waking is being considered..

Apart from this, the ovarian condition of a marmot about to go to sleep does not correspond with the syndrome of hypopituitism. Nevertheless, the observable changes take place in the chromophile cells, and it may be that normally these are principally concerned in inhibiting the conversion of sugar into fat, whilst others are concerned with the ovarian activity. If one bears in mind the reservations that have been made concerning the importance of histological changes, one gets a very pretty picture of a gradually decreasing pituitary activity as fat is put on in preparation for the winter sleep.

There is one other component of the internal environment with which the pituitary is largely concerned, and that is with the water balance in the body. Since this question appears to be of very great importance in the phenomena associated with prolonged inactivity, a special section will be devoted to it.

(c) *The water balance.*

Most observers are agreed on some change in water content occurring during hibernation, although the change may not always be in the same direction. The most uniform, and probably the most valuable, work has been performed on insects. This is largely because it is easy to get a large number of insects to analyse at a time, and also because it is easier to desiccate the entire animals.

In agreement with the workers on insects Bellion (1894) found that there was a decrease in the water content of snails during the winter sleep. Also he discovered that in summer desiccation caused the animals to seal up their shells and become dormant—a result identical with that of Breitenbecher (1918) on the potato beetle. Bodine (1923) found a similar decrease in the case of a hibernating grasshopper. For mammals Dubois (1896) finds indications of a decreased water content in the marmot from figures obtained from a study of its blood. This result confirms that of Aeby (1874) for the tissues of the same animal. The dissentients from this rule are Gradinesco (quoted by Athanasiu, 1909), who finds an increased water content in the frog, and Rasmussen (1916 *d*), who finds no alteration in the size of the red cells, or in the specific gravity of the blood of the marmot. It should be mentioned that the size of red cells varies with the carbon dioxide content, and there is no indication that Rasmussen has taken this into account (*vide supra*). It is true that such changes are by no means invariable, but the two values should be correlated.

Two aspects of the effects of changes in water content are possible, one of which deals with the effects of a decrease in this value, the other with the effects of an increase. The former is dealt with by Bayliss (1924) and by Breitenbecher (*loc. cit.*), who show how a decreased water content aids in maintaining inactivity. Rowntree (1922), on the other hand, shows how, in the case of mammalia at any rate, a decreased water content can be associated with fever.

Bayliss deals more especially with the importance of a eutectic, or one should probably say, eutectoid state. He points out that not only are many organic substances far more stable if dried, but that bacteria are more hardy in this condition, and also that there is evidence that certain seeds and spores have a decreased water content. He fully realises that changes in temperature probably exert their

deleterious effects by destroying the architecture of the cells. Thus we may imagine that the solidification of some of lipid interface at a low temperature would disarrange irreparably the equilibrium in the heterogeneous system of protoplasm. He points out that if protoplasm is cooled *quickly* to a temperature of about 30° C. and desiccated, it is not apparently damaged structurally. A difficulty is encountered when an attempt is made to re-introduce water. It is probable that the analogy drawn between this phenomenon and the synchronous changes of state occurring in an alloy at the eutectic point is too literal. For one thing, the adaptive changes of protoplasm to changes in temperature tend to be gradual, whilst to obtain synchronous crystallisation one must cool rapidly to the eutectic point. Nevertheless, that some similar process is at work seems quite probable.

Breitenbecher deals rather with the possible slowing effect of lack of water on chemical reactions involving hydrolysis. He gives several very interesting examples of the protective effects of a partial dehydration, such as that of Hunter and Hinds (1904), which shows that for the Mexican cotton weevil moisture is far more harmful than a drop in temperature. He himself showed that the potato beetle when in its active state, *i.e.* with its full water content, perished if buried. However, when desiccated it may be buried for long periods without harm. Incidentally an interesting comparison with this experiment is that of Pallas (quoted by P. Bert, 1870), who found that the hamster began to hibernate when buried. As we have already seen, the potato beetle must dehydrate itself before retiring to its winter quarters.

As has been already said, there is no unanimity as to changes in water balance that may occur during mammalian hibernation.

From observations made on mammals that do not hibernate, it appears that withdrawal of water from the tissues causes an increase in metabolism. Thus Crendall (1899) believes that the febrile attacks of infancy are due to lack of water. Heim and John (1910) give evidence in support of the idea that the fevers that follow intravenous injections of salts are produced by a withdrawal of water from the tissues. The effect of a withdrawal of water from the system in which chemical reactions are occurring might be in the direction of either an increase or a decrease in the velocity of the reactions, depending on whether it was playing the part of an active constituent, or merely of a passive medium. Further, the precise condition of water in protoplasm is difficult to state. Rowntree (*loc. cit.*) mentions a distinction between free and fixed water, but it must be confessed that it is not at all clear how to define the two conditions in protoplasm. Some animals can lose very large amounts of water without harm. Thus Breitenbecher states that the potato beetle can lose 50 per cent. of its water, a frog 39 per cent. and the June bug 15 per cent. without harm. In the case of mammalia, Engels (1904) has shown that a large amount (over 60 per cent.) of water lost by the organism is given up by the muscles. Durig (1901 and 1903) showed that the loss of water by these tissues may be in the region of 10 per cent., without there being any disturbance of function or structure. It is possible that part at any rate of this water, that may be lost without harm, is used merely as a menstruum for the passage of metabolites, etc., to and

from the cell and does not possess any structural importance whatever. We have seen, however, that the loss of water in the potato beetle has very profound metabolic significance. In the case of higher animals the results are far more difficult of interpretation; even in cases where no change in the amount is detectable during hibernation the metabolic modifications may be brought about by a change in its distribution. Thus Dubois has noticed that the marmot may have ascites during its winter sleep. Whether the pituitary or any other endocrine influences this distribution is an interesting problem.

Now, important though these constitutional changes are, the fact must not be lost sight of, that they are produced primarily as a response to environmental changes. And in connection with this, Breitenbecher has some very interesting observations on the reciprocal action of the internal and external environment in the case of the potato beetle. He finds that a normal insect possesses a positive heliotropism and a negative geotropism. After dehydration, which, it must be remembered, happens either by virtue of the animal's own activity if under natural conditions, or by artificial means, these tropisms are reversed. These changes obviously cause the animal to burrow and seek its winter quarters under the appropriate circumstances. A dormant animal when dug up may crawl about actively, but the tropisms remain the same till the air is moist.

The internal environment of higher animals does not, in general, reflect the external environmental changes so literally as those of less complex types; it is, therefore, worth while to study the type of nervous system possessed by a hibernating mammal in a section by itself.

III. THE NERVOUS SYSTEM OF A HIBERNATING MAMMAL.

Anybody who has dealt with hibernating mammals must know that an animal so lethargic as to appear lifeless may be awakened if manipulated at all carelessly. Further, as Mangili (1808) has shown, a bat will wake up if the cold becomes too severe. These observations by themselves ought to make anybody suspicious of statements of severe structural modifications as reported by certain people. Levi (1898) describes profound alterations in the staining reactions of the cord of the frog, but failed to find them in mammals. Legge (1899), however, found a similar diminution in Nissl's granules and a generally basophil reaction of the cytoplasm in mammals. There are many other similar observations (see Athanasiu, 1909, and various other reviews), among which those of Querton (1898) are of some interest; he describes reaction of the axons similar to that which may or may not take place during normal sleep. Finally Rasmussen and Myers (1916) failed to find any such changes in staining reaction, an observation which is not surprising, considering how easily reversible the lethargic state is. Before leaving the histology of the nervous system, let us note the researches of Marinesco (1906) on the comparison of the neurofibrillar structure of warm- and cold-blooded animals. He finds that mammals in general have far finer neurofibrils than poikilotherms, and that this difference is maintained during hibernation, although he indicates that at tempera-

tures of about 10° C. the fibrils of the former animals tend to hypertrophy. How far neurofibrils are representative of the true structure of the nerve cell has been subject to considerable criticism.

The withdrawal from the environment by mammals is not so complete as in some lower animals, and so one finds a truly remarkable sensitivity of the nervous system even at as low a temperature as 4° C. As is well known, certain animals, e.g. hedgehog and marmot, roll themselves up into a ball whilst asleep. How the tone of the muscle responsible is maintained is a problem by itself, but on stimulating a hedgehog quite lightly with the finger, the ball is tightened. Marshall Hall records that a hedgehog, though very deeply asleep, will swim if thrown into water. Merzbacher (1903) records that a bat will drink on being brought into a warm room long before its temperature has risen to that of its surroundings. Forel (1887) made some interesting observations on a dormouse which was lethargic in summer; he showed that the animal could hang some time by one paw till it was fatigued, and then change it without waking. Doubtless many other observations of similar nature have been made, both published and unpublished; they all go to demonstrate the remarkable adaptability of the nervous system to changes in temperature from 37° to 4° , although of course the activity is enormously reduced. Valentin gives the velocity of the impulse as 1 metre per second. There is one reflex obtainable from a bat (Merzbacher, 1903) which is worth mentioning, since it suggests a parallel to Breitenbecher's results. It is a postural reflex, and consists of a rather complicated series of righting reactions that occur when the animal is placed on its back. This reflex is only elicitable when the animal is hibernating. Merzbacher has shown that the cerebellum is not necessary for it, and by the method of serial sections has come to the conclusion that its path is through the medulla oblongata.

As was suggested in the opening section of this paper, a primitive type of nervous system is to be expected in a hibernating mammal. There is certainly evidence that this is the case; thus Merzbacher (*loc. cit.*) finds that effects of decerebration on a bat resemble those one finds in the case of a bird. Dubois (1893, 1894) finds that the rise of temperature during the waking of a marmot is not abolished by decerebration. He localises the respiratory centre, and that controlling the rise of temperature in the anterior part of the aqueduct of Sylvius, and in the side of the floor of the third ventricle. However, he finds that the rise in temperature on waking is not entirely abolished by sections as far down as the fourth cervical vertebra.

Altogether, the nervous system of these animals offers a most interesting stage in the evolution of the mammalian nervous system, and should well repay study, especially for its thermal and postural reactions.

IV. THE CONCLUSION OF HIBERNATION.

How far a state of lethargy is uninterrupted varies from animal to animal. Thus some insects will wake up if the weather becomes warm, bats sometimes fly about in their winter quarters, and bears are never very profoundly asleep. Many animals wake up to feed on stores they have taken into their winter quarters with them.

Bodine (*loc. cit.*) has shown this to be true for certain grasshoppers, a property shared by them with the Russian peasants described by Volkow. Dubois lays stress on the importance of an accumulation of carbon dioxide as a cause of waking, but it seems probable that equilibrium is attained within a day or two of the onset of lethargy between the production and excretion of the substance concerned. (*Vide supra*, and Pembrey and Gorer's paper to be published shortly.) However, the accumulation of urine and faeces very probably tends to cause the interruptions of winter sleep that have been observed for marmots and other animals in the coldest weather.

The influence of moisture is probably of more importance during aestivation than during hibernation. Krogh, indeed, dismisses aestivation as being purely a result of variations in humidity, and does not seem to think it has much in common with hibernation. But the behaviour of two such closely related animals as the European hedgehog and the Madagascan tenrec suggests, apart from any of the other experiments already quoted, that there must be several points in common between the two phenomena.

The effects of injecting various substances are probably not really representative of the natural course of events. They probably act like any other stimulant, and their effect of enhancing the activity of the peripheral tissues must give rise to a stream of impulses to the central nervous system, even if they do not affect these tissues directly, as Merzbacher has succeeded in doing by painting the cerebrum with creatinine.

An interesting problem is the determination of the source of energy for raising the temperature of mammals when they wake. Pembrey considers that the shivering is largely responsible for this rise. Dubois believes that it is very largely due to the activity of the liver. It should be mentioned that the rise in temperature takes place far more rapidly at the head than at the hind end of the animal. A marmot may be paraplegic when the temperature in the mouth is quite high.

The various quotients have already been mentioned, and one must agree with Krogh that, considering the upset of gaseous equilibrium that must be occurring as the temperature changes, together with possible qualitative changes in metabolism, deductions from the quotient or indirect calorimetry are unreliable (not to mention the unknown rate of loss of heat), and that experiments on direct calorimetry are to be desired.

In the preceding pages an attempt has been made to give a more or less continuous account of the physiological conditions of an animal during a temporary withdrawal from its environment. The gaps in our knowledge are enormous, and the author has attempted to show how an answer to any of the many questions this problem sets would throw light, not only on the physiology of lethargy, but upon almost any of the more general problems of the mechanism of adaptation.

V. SUMMARY.

1. It is stated that hibernation is but one manifestation of a property very widely spread amongst living organisms, *i.e.* that of withdrawing from an unfavourable environment. It is urged that comparison of hibernation with other analogous conditions would be valuable. Evidence is given that somatic changes occurring for various unknown reasons may cause the assumption of lethargy at an unusual time. Therefore somatic changes are as important in determining the cause of hibernation as environmental changes.

2. The metabolism of hibernating animals, which is the lowest metabolism required to maintain the existence of protoplasm, is discussed. The various values obtained in connection with the respiratory exchange are mentioned. It is pointed out that the chief points of contention concern themselves with the possible effects of temperature on the solubility of CO₂. From experiments covering long periods it is urged that the observed phenomena cannot be explained in this way. The adaptation to changes in temperature is discussed to a certain extent.

3. The governor of metabolism amongst the higher animals apart from the nervous system is the endocrine system. The value of various histological findings, and of various injections, is discussed. It is pointed out that lack of structural modification does not necessarily eliminate the possibility of functional modification. Special attention is paid to the importance of the pituitary.

4. The effects of varying concentrations of water on the metabolic processes are discussed. It is also indicated how the water content can modify the behaviour of an animal, and that a reduction of it may have opposite effects in various cases.

5. Observations are given which demonstrate the remarkable adaptivity, and primitive structure, of the nervous system of hibernating mammals. Particular stress is given to the comparatively slight effects of decerebration on the heat regulative and postural reactions of such animals.

6. The various internal and external stimuli tending to terminate lethargy are mentioned, as well as the phenomena connected with the actual return to activity. Emphasis is laid on the need of direct calorimetry to elucidate this problem in the mammalia.

Finally I should like to express my very sincere thanks to Prof. Pembrey for the help he has given me in the preparation of this review, and to Mr T. J. Evans for much valuable criticism.

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THE PROBLEM OF GRAFT HYBRIDS AND CHIMAERAS

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(Received May 3, 1930.)

(With Five Text-figures.)

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I. INTRODUCTION.

GRAFTING seems to have been practised from time immemorial by nations skilled in horticulture. According to Daniel (1923) the practice of grafting was carried on by the Chinese many thousands of years before the Christian era, and is also said to have been known to the Phoenicians. That it was known to the ancient Greeks is evidenced by the statement of Theophrastus, the father of botany as he has been called, in his *Enquiry into Plants* (Book II, par. 3) that "with those plants with which it is possible, shoots from the boughs should also, they say, be planted, some being set on the trees themselves as with the olive, pear, apple and fig." Virgil too alludes to the possibility of grafting, but allows his fancy to carry him away when he writes (*Georgics*, Book II)

Vigorous apples are grown on the barren plane,
A beech bears chestnuts, a mountain ash the silver shine
Of pear-blossom, under the elm have acorns been crushed by swine.

Dr A. S. Way's version.

Only plants of comparatively near relationship seem to be able to become permanently united by grafting, the process being facilitated in such cases by the similarity of their tissues. Experiments as to the limits of difference of stock and scion do not seem to have been carried out systematically, though as a result of the work of Voechting (1892) and Daniel (1918) we know that herbaceous as well as

woody plants can readily be grafted and annuals and perennials can be joined together.

Daniel, as a result of his wide experience in grafting, holds, as is well known, the belief that the stock may influence the scion or *vice versa* (see Daniel, 1899, 1922), and this feature, if it really exists, must be taken into account when we consider the question of graft hybrids. In general, the influence of stock on scion is denied by authorities on grafting like Winkler (1912), except in connection with the transmission of diseases such as infectious chlorosis which has been studied by Baur (1904, 1906) and others in the family of Malvaceae, a group of plants very prone to this ailment, though occurring, as Baur has shown, in plants belonging to other families too. This phenomenon of the transmission of variegation from scion to stock in the case of a jessamine was first noticed about 1700 by Watts in Kensington, and described by Blair (1720). Later Lemoine observed that slips of a variegated form of *Abutilon striatum* distributed by Messrs Veitch as *Abutilon Thompsoni*, when grafted on a green stock, transmitted the peculiarity of its foliage. Morren (1869) and Lindemuth (1878) made further experiments and investigations in elucidation of this phenomenon, but it was left to Baur to clear up the difficulties surrounding it in a series of interesting communications (1904, 1906). Apparently the disease can only be transmitted by grafting a living shoot or even a single leaf of the variegated form on a green stock. The expressed sap of the leaf if injected into a green plant is without effect. The "virus" which Baur supposes to be the cause of the disease is, according to his interpretation, not an organised body like some of the known viruses, but an organic substance continually formed in the yellow portions of the variegated leaf. For if these yellow portions are cut out systematically, the disease can be stayed and the plant becomes wholly green again. This infective substance passes down or up in the cortex and phloem of the plant, *i.e.* along living tissues. It is of interest, however, to note that it does not pass into the seeds, for offspring raised from seed are always green. Some species, *Abutilon arboreum* for instance, are immune to this virus, yet their cells can be penetrated by it. For if a double graft is made, *i.e.* a susceptible form is grafted on the immune form, which itself has been grafted on a variegated stock, the latter transmits its variegation through the completely green *Abutilon arboreum* to a susceptible scion of *A. avicinnae* for instance.

In this connection it is of interest to note that Dr Salaman (1930) has recently shown that, in the case of potatoes infected by the virus causing crinkle, this disease can be transmitted through a portion of the stem of *Datura*, which is itself immune to the disease. He has also been able to demonstrate that the virus becomes attenuated by passing through *Datura*. We are probably dealing, therefore, with a different kind of virus in these two cases, one organised and the other only an organic substance of the nature of a toxine secreted by the diseased yellow patches of the leaf, but at the same time, as Baur suggests, capable under certain conditions of increasing in amount by detaching from other organic compounds substances similar to itself in consonance with Ehrlich's theory of toxines. Plants belonging to other families may suffer from infectious chlorosis. Baur has been able to demon-

strate such cases in *Laburnum*, *Sorbus*, *Ptelea*, *Fraxinus*, *Euonymus* and *Ligustrum*, the chlorosis not always showing itself as variegation, but sometimes in the form of yellow-green leaves like "aurea" varieties.

This account of infectious chlorosis may seem to have nothing to do with graft hybrids, but it represents an effect of the scion on the stock, which was taken at one time to demonstrate the interaction of the two symbionts. Many other instances of interaction have been described from time to time, but they have either not stood the test of crucial experiments such as Baur undertook, or may be explained in other ways. Among these cases may be mentioned the one described by Edler (1908), who grafted sugar-beets on beetroot stock. The seeds collected from the inflorescences of the grafted sugar-beet yielded some seedlings with a distinct red colour: Baur (1919) indicates, however, that even from ungrafted sugar-beet one can occasionally obtain distinctly red seedlings, which may be due to crossing at some previous time or to other causes. Graft hybrids of the nature described by Edler are problematical, though we shall have to consider some others supposed to be caused directly by grafting.

Daniel (1914), in his account of "l'hybridation asexuelle," emphasises, as the sub-title of his paper indicates, the specific variations which arise in grafted plants. No botanist has probably had greater experimental experience in grafting plants of a great variety of families, and Daniel has come very definitely to the conclusion that there is a great amount of interaction between stock and scion. He has not only described many apparent variations thus induced, but his experiments lead him to believe that such variations are inherited by the offspring of the graft (1895). He believes that new varieties of plants can thus be produced, and has developed a method of grafting, *le greffe mixte* (1897), by means of which the influence of the stock on the scion may be increased and graft hybrids might be obtained. In this special form of grafting Daniel recommends that a shoot of the stock just below the insertion of the scion should be allowed to develop, instead of cutting it back, as is the usual practice in grafting. By leaving a shoot of the stock to develop, the upward passage of sap is promoted and also a downward passage of elaborated sap from the shoot to the stock will be induced. Thus, he states, a blending of characters will take place. By leaving only the scion to develop, the character of the latter alone is maintained and this is, of course, the object of normal grafting where the maintenance of some choice form is desired. The purpose of Daniel's method of "mixed grafting," on the other hand, is to obtain new variations by a blending of characters not unlike new varieties obtained by ordinary hybridisation. Daniel's views on the specific influence of stock on scion and *vice versa* have, however, not met with general acceptance. The more usual view is that, apart from the authenticated cases of the transmission of infectious chlorosis mentioned above, in which the scion transmits the disease to the stock, any modifying effect of the stock on the graft must be of extraordinary rare occurrence, otherwise the practice of grafting would have lost its *raison d'être*.

That, however, there have occasionally arisen on grafted plants branches which have borne leaves and flowers in character intermediate between those of stock and

scion, as well as reverions to the two plants united by grafting, is beyond doubt. Whether we should regard such developments as graft hybrids, interpreting them as having arisen by the union of two vegetative cells of stock and scion, or whether they consist of the still unaltered tissues of the two component plants closely associated together, and forming what Winkler (1908) has termed a chimaera, remains still to be settled for each individual case. Hence the problem of graft hybrids still exists. According to Daniel (1923, p. 175) the first recorded case of the occurrence of a graft hybrid is that mentioned in the fifth century B.C. by Feing-Lu, writing under the pseudonym of Pao Tscheou Kon, who describes a tree occurring in China formed by grafting a peach upon a plum stock. This tree produced fruits exhibiting a mixture of the characters of the two components of the graft. Of course the correctness of this observation cannot be vouched for, and the same is true of other presumptive graft hybrids which have been recorded from time to time in horticultural literature.

II. THE BIZZARRIA ORANGE.

Apart from this very early record which cannot, of course, be verified, the first authenticated case, which has been the subject of many investigations, is the famous bizzarria orange which was raised in Florence in 1644. It is briefly referred to by Darwin (1888) in his discussion on the subject of graft hybrids, and is described for the first time in English in Volume II of the *Philosophical Transactions of the Royal Society* (1731, p. 659), the description being from the account given by Nati (1674). The passage runs as follows:

The first Original of this Tree was by inoculating Orange upon a Citron-Lemon Stock so that by the Marriage of these Trees repeated for many Years, it was come to pass, that by the Closeness of the Inoculation, whereby in the Length of Time the mixed Nature of both Trees was grown together, which the different Juices, permeating the common Fibres had for a long Time nourished, there emerged a Germen or Graft perfectly retaining the Nature and Species of both; into whose different Branches when sometime one, sometime both kinds of Juices did pass, it produced on one of those Branches a mere Orange, on another a Citron-Lemon, on a third a Citron-Lemon-Orange, and even on the same Branch all the sorts of this Fruit together.

The gardener who raised this tree in Florence in 1644 declared that it had been raised as a seedling and then grafted. The graft having perished, the stock sprouted and produced the bizzarria, the characteristic of which was that it produced at the same time leaves, flowers and fruits both of the bitter orange and of the citron of Florence, and also compound fruits with the two kinds blended together or segregated in various ways. This curious tree has been propagated by cuttings, which have retained this habit. Many authors have supported the view that this bizzarria orange is a graft hybrid, but others have considered that the stock was probably not a pure type but a seed hybrid, since the various species of *Citrus* hybridise readily.

Cramer (1907), for instance, in his exhaustive treatise on bud variation is inclined to regard this case (p. 309) as one which is to be explained by vegetative segregation of a normal seed hybrid. But Cramer is exceedingly sceptical of the

occurrence of any graft hybrids, and very critical of the cases cited in horticultural, and even scientific publications, a large number of which he passes in review. Even *Cytisus Adami* and *Crataegomespilus*, now generally admitted to have originated by grafting, he considers doubtful. He is no doubt influenced by the large number of cases of vegetative segregation of seed hybrids known to him.

In dealing with the bizzarria orange one is more inclined to accept the views of Strasburger (1907) and Frost (1926), both of whom have examined critically the behaviour of plants descended from the original bizzarria stock and have a knowledge of other cultivated forms of *Citrus* and their hybrids. Strasburger, who was able to consult many of the earlier and somewhat inaccessible accounts of the bizzarria *Citrus* and records of its cultivation, gives a detailed account of its history and discusses the probability of its being a seed or graft hybrid and on the whole favours the view that the seed hybrid nature is the more probable. In his very critical account, which gives a very complete summary of the available older literature on the subject, he refers to the fact that two contemporary Florentines, the doctor Pietro Nati (1674) and Georges Gallesio, had secured from the gardener of the Panciatichi Gardens in Florence the confession that the bizzarria had arisen spontaneously from a seedling which had been used as a stock for a grafting which, however, had not been successful. The peculiar bizzarria had arisen, according to this statement, spontaneously as an adventitious bud from the swelling, where the unsuccessful scion had been inserted. They concluded, and this conclusion was accepted by Penzig (1887) who has carefully reviewed all the earlier accounts of this peculiar plant, that the seedling which was used as stock was itself a seed hybrid and the segregation of parental characters, sometimes partial and sectorial and sometimes complete, was such as may occasionally be observed in other hybrids. On the other hand, the failure of the inserted scion to develop does not preclude the possibility of some of its tissue having remained in a living condition, and having united in some way or other with that of the stock. Indeed, the history of a later graft hybrid, *Cytisus Adami*, is a close parallel to that of the "original" bizzarria. I speak of it as original, since Strasburger mentions the fact that other bizzarria oranges are stated to have arisen in other gardens, some of them according to definite statements also from grafting. Strasburger thinks it very unlikely that such a rare and somewhat problematical occurrence as the union of two vegetative cells should have arisen repeatedly, and is, therefore, more inclined to accept the sexual origin of these bizzarrias, all the more as in some cases and indeed according to Penzig's account in the original bizzarria, reversions to three kinds of *Citrus* seem to occur. He sums up his account by the following sentence: "Während die Vorstellung dass eine mehrfach zusammengesetzte Bizzarria durch vegetative Kernverschmelzungen hätte entstehen können auf fast unüberwindliche Hindernisse stösst, bietet die Annahme dass eine solche *Bizzarria* ein zusammengesetzter sexueller Bastard sei, keinerlei Schwierigkeiten." He adds, however, that one must then assume the possibility in sexual hybrids of such segregation of characters as is shown by the bizzarria orange and other presumptive graft hybrids.

In a later publication (1909), after the proof by Hans Winkler in 1908 that

graft hybrids between *Solanum nigrum* and *Solanum lycopersicum* were obtainable experimentally, Strasburger revised his views and indicated the new position he took up on the question of graft hybrids. He admits that the segregation of characters which had been observed in the bizzarria and other graft hybrids, is not in agreement with a possible sexual origin of the same, and says: "ich selbst bemühte mich, wie ich jetzt einsehe, mit Unrecht ab, diese Abweichungen ihrer principiellen Bedeutung zu entkleiden." The chief importance of these frequent reversions and partial segregation of characters into sectorial and other chimaeras he now ascribes to the fact that, in such graft hybrids as the bizzarias, the individuality of the parental cells has not been lost by any cell fusion, but is able to express itself from time to time in definite parental cell complexes, sometimes lying side by side in the same organ, sometimes giving rise to complete parental shoots. He does not, however, accept the periclinal chimaera theory of Baur (1909), according to which the parental tissues form two closely connected layers, one surrounding the other, forming a core and a covering skin, but assumes a more intimate and irregular arrangement of the tissues derived from the stock and the graft respectively, to which composition he gives the name of hyperchimaera, comparing it in its morphological unity and physiological behaviour to a lichen rather than to a seed hybrid. But the components being nearly related, the union is more intimate, and protoplasmic connection between adjacent cells leads probably to the transmission of some modifying stimulus.

Frost (1926), who has a wide experience of the cultivation and crossing of various species of *Citrus*, has come to the conclusion, as have other workers on the Californian *Citrus* plantations, such as Shamel, Scott, Pomeroy and Dyer (1920), who are familiar with the considerable amount of bud variation in the cultivated species of *Citrus*, that most of these cultivated forms have an extremely heterozygous genetic constitution. Frost considers that this causes a liability to somatic segregation, possibly in single cells, either by general mutation or more probably by differential mitosis. To produce from these groups of cells recognisable bud variations requires bud formation in the variant tissue. This may cause the occurrence of sectorial, periclinal or mixed chimaeras, which may show themselves either in individual leaves or in the flowers and fruits. If these chimaeras arise in the tissues of a seed hybrid of a heterozygous variety, he would call them *autogenous* chimaeras in contradistinction to those produced directly by grafting, which he designates as *synthetic* chimaeras. Both kinds may, according to Frost, occur in *Citrus*, and he thinks it is probable that the historical example of the Florentine bizzarria orange may have arisen by grafting. Since we know from Winkler's experiments that chimaeras can be produced by grafting, there is no reason to doubt the account given concerning the origin of the bizzarria orange.

III. *CYTISUS ADAMI*.

The second historical instance of a graft hybrid, or we should perhaps call it a chimaera, *Cytisus Adami*, is, after an equally considerable amount of speculation and experiment, less in doubt. According to the records a nurseryman, M. Adam

at Vitry near Paris, budded in 1825 a shield of the small tufted *Cytisus purpureus* on the common laburnum, *Cytisus Laburnum* or, as it should now be called, *Laburnum vulgare*. The bud seemed to die back, but from the region of its insertion a strong shoot developed later. This shoot assumed an arborescent habit, very different from the more prostrate form of the purple *Cytisus* and subsequently produced flowers in form and colour exactly intermediate between those of stock and scion respectively. The foliage, too, was of intermediate size. It looked, therefore, as if in this instance a true graft hybrid had been obtained, and as such it is referred to by Darwin (1888, Vol. I. p. 416). It has been frequently described both in horticultural and scientific literature. Both Gaertner (1849) and Focke (1881) in their books on plant hybrids discuss the possibility of the occurrence of graft hybrids, and give details of the peculiar occurrences observed in *Cytisus Adami*. While recognising the difficulties involved in the acceptance of such peculiar and abnormal plant structures, they are inclined to accept the possibility of the existence of vegetatively produced hybrids. One of the peculiar features of *Cytisus Adami*, which is now grown grafted on *Laburnum* stock in most botanical and many private gardens as a botanical curiosity, is the tendency of the tree to revert back in some of its branches to the two components of the symbiont, producing some ordinary inflorescences of the *Laburnum* and some groups of the paired flowers of the purple *Cytisus* together with the appropriate foliage, while at the same time a third type of inflorescence with flowers of intermediate shape and colour is borne on the same tree. This obvious segregation of characters can show itself also on single inflorescences, which may bear two kinds of flowers sometimes sectorially arranged or, indeed, single petals may be longitudinally differentiated. This phenomenon has been fully described by Alexander Braun (1853) in his Rejuvenescence and also by Beijerinck (1900), the latter of whom has made many experiments to determine the possible causes of the reversion of *Cytisus Adami* to *Laburnum vulgare* and *Cytisus purpureus* respectively. Cramer (1907), on the other hand, who repeatedly refers to *Cytisus Adami* and its peculiarities and is, in general, very sceptical as to the occurrence of graft hybrids, considers that this segregation of characters is in no way different from similar occurrences in seed hybrids. In the case of *Cytisus*, contrary to the interbreeding of the species of *Citrus* concerned in the bizzarria orange, we know from repeated experiments by Caspary and Darwin that it has been impossible to obtain a seed hybrid from *Laburnum vulgare* and *Cytisus purpureus*. Curiously enough, both species have produced hybrids with other species of *Cytisus*. Thus *Cytisus purpureo* × *elongatus* and *C. Alpino* × *laburnum* have been obtained by Wettstein (1891). These hybrids differ from *Cytisus Adami* in the fact that their pollen grains are badly formed, nearly 85% being abortive, while those of *Adami* are fairly well formed. Whether this difference is of some significance or not, it inclined Caspary (1865) to accept the recorded evidence in favour of the origin of *Cytisus Adami* by vegetative means, i.e. by grafting. The ovules of *Cytisus Adami*, as has been shown by Tischler (1903), are curiously malformed, the nucellus together with the inner integument projecting considerably beyond the outer integument. A similar deformed ovule of *Laburnum vulgare* was discovered by Buder (1911) and

is believed by him to be due to pressure on the ovule during development. The normal condition for *Cytisus Adami* might, therefore, be due to the pressure of the external upon the internal component of the plant.

Macfarlane (1891), in his very thorough examination of the minute structure of plant hybrids, refers to a striking difference observed by him between the minute structure of the various organs of *Cytisus Adami* and that presented by all the seed hybrids he had examined. In the latter he found that such details as size and shape of the cells, and even the size of the nucleus, were intermediate between the corresponding features of the two parents, whereas in the case of *Cytisus Adami*, to use Macfarlane's words, "the very striking resemblance which the epidermis of the hybrid portion has to that of *C. purpureus*, not only in the general structure of the cells, but in the size and structure of the cell nucleus, the distribution of the stomata and specially of the hairs, would seem at first sight to prove that the hybrid portion was wrapped round, so to speak, by an epidermis of *C. purpureus*." This description, given 18 years before Baur advanced his theory of the chimaeric nature of graft hybrids, does great credit to the accuracy of Macfarlane's observation, and to the suggestiveness of his comparison. Macfarlane, however, felt himself compelled to assume that a union of nuclei had taken place at the juncture of stock and scion in view of the importance played by the nucleus in cell life, and also by the close resemblance which the flowers of *Cytisus Adami* have to those of a seed hybrid, though in a further paragraph he ventures the suggestion "that it may be even that the contact of the two sets of cells of stock and graft causes a physiological stimulus analogous to that of fertilisation." Various investigators had taken up the question of the structure of graft hybrids thus opened up by Macfarlane, of whom Fuchs (1898), Laubert (1901) and Noll (1902, 1907) were the most important. All three accepted the evidence of the production of *Cytisus Adami* as originating from a graft, and of the composition being similar to that of a seed hybrid. In his earlier paper Noll (1902) is prepared to accept the view that a fusion of vegetative cells had taken place. It became, therefore, important to ascertain whether in this case a fusion of the nuclei of the vegetative cells had occurred. Observations made by others concerning the peculiarities of *Cytisus Adami* prevented him from coming to any other conclusion than that the cells in the growing points of *Laburnum Adami*, as he calls it, contain nuclei which unite the chromosomes of *Laburnum vulgare* and *Cytisus purpureus*.

This problem was investigated by Strasburger (1906, 1907). Macfarlane had already pointed out differences in the size of the nuclei in the two species of *Cytisus* and in the different cells of the presumptive graft hybrid. It was now important to see whether the number of chromosomes of *Cytisus Adami* was double that of its constituent species, as Weismann had already suggested in 1892 it should be if it were a true graft hybrid, or whether any of its cells contained two separate nuclei, which might be possible. It turned out, however, that the cells were all uninucleate and the chromosomes of *Cytisus Adami* in the somatic cells were 48 in number, as they are in the two grafted species. Of course, a fusion of two nuclei of vegetative cells of the constituent symbionts might still have taken place, and a reduction of

the nuclear chromosomes might have taken place by some sort of auto-regulatory process as suggested by Nemec (1904), in the case of the vegetative cells in which he had increased the number of nuclei by the action of chloral hydrate. Strasburger, who had repeated Nemec's experiments, was in disagreement with him. He found it impossible, on the strength of his own investigations and the observations made by others concerning the peculiarities of behaviour of *Cytisus Adami*, to come to any other conclusion than to assume that the cells in the growing points of *Laburnum Adami*, as he calls it, contain nuclei which unite the chromosomes of *Laburnum vulgare* and *Cytisus purpureus*.

The next step forward was the suggestion made by Baur (1910) that graft hybrids were of the nature of periclinal chimaeras, a term he had suggested for a growth formed by two genetic tissues, one forming a central core and the other a covering skin. This type of development Baur (1909) found to be the cause of the formation of white-margined varieties (var. *albomarginatae hort.*), such as we get in *Pelargonium zonale*, and he extended this conception to graft hybrids. Of course it is obvious that, though the term periclinal chimaera may be used in both cases, the origin of the white-margined varieties takes place, as we shall see later, by a sexual process, whereas *Cytisus Adami* had resulted from grafting. Nevertheless, the comparison offers many suggestive features. According to Baur's view, *Cytisus Adami* consists of a core of *Laburnum* tissue surrounded by an epidermal layer of *Cytisus purpureus*, this latter layer being carried up around the adventitious bud formed at the point of junction of stock and scion, a suggestion which brings it into line with Macfarlane's observations. The coloured figure in Baur's text-book (Pl. X) shows most clearly how this interpretation fits the flower colour of the three forms. *Cytisus purpureus* has a purple sap in the epidermal cells of the petals, while the *Laburnum* has yellow coloured plastids in this and in the underlying cells. In *Cytisus Adami* the epidermis has purple sap of *C. purpureus*, while the underlying cells have yellow plastids of the *Laburnum*, hence the yellowish brown tint of petals. The brown marking of the *Laburnum* petals belonging to the sub-epidermal layer is also present. Baur's periclinal hypothesis led at once to renewed and fruitful investigations. The most important of these, as far as *Cytisus Adami* is concerned, were those of Buder (1910 a, 1911). These were entirely confirmatory of the periclinal hypothesis, and the second and more important brought to light several new facts, while Buder was able to confirm those recorded by Macfarlane, whose first description of the structure of *Cytisus Adami*, cited above in italics, certainly supports Baur's hypothesis. Among the new facts mentioned by Buder are those connected with the formation of the cork in the stem. In *Cytisus purpureus* the cork is epidermal, in *Laburnum vulgare* it develops from the second cortical layer. In the *Cytisus Adami*, which is to be looked upon as a periclinal chimaera with the epidermis of *Cytisus purpureus*, two cork layers are formed, both the epidermal cork of *Cytisus purpureus* and the cortical phellogen of *Laburnum vulgare*. At the same time Buder shows that, though genetically distinct, the tissues of the two parental plants are physiologically united, for there is protoplasmic continuity between the cells of the epidermis and those of the sub-epidermal layer. For the detection of the purple *Cytisus* constituent

Buder relies in many instances upon the deeply staining tannin compounds of the cells of this plant. Such tannin compounds are characteristic of the epidermis of *Cytisus Adami* and enabled him to detect many partial reversions in the leaves of the component symbionts. Another interesting experimental verification of the periclinal chimaera theory by Buder was the production of reversions to the *Laburnum* stock by wounding the epidermal cells of a bud. In that case the *Laburnum* core regenerated the apical tissues, and so the whole shoot became a pure *Laburnum*. Reversions to *Cytisus purpureus* must occur by proliferation of the epidermal layer to form a completely "purpureus" apex.

Something should be said here concerning the seed reproduction of *Cytisus Adami*. As Darwin had already stated, the reversions from *Cytisus Adami* are perfectly fertile, and he and others raised seedlings which did not differ at all or only very slightly from the normal species. *Cytisus Adami* itself proved to be sterile, partly no doubt owing to the malformation of its ovules. Caspary (1859) had already discovered the abnormal formation of the ovule, and Tischler (1903) had drawn further attention to it. Nevertheless, occasionally though rarely, it has been possible to obtain fertile seeds from *Cytisus Adami*; as they have been found on old racemes by both Noll (1907) and Hildebrand (1908). The latter reported that they had given rise to pure *Laburnum* seedlings. This phenomenon, at first sight puzzling, is in agreement, however, with Baur's interpretation of this graft hybrid as a periclinal chimaera. For since the reproductive cells of the Angiosperms are formed from sub-epidermal cells and since, according to Baur's views, only the epidermis partakes of the nature of *Cytisus purpureus*, while all the underlying cells are pure *Laburnum*, it follows that both the egg cells and the pollen grains of *Cytisus Adami* are pure *Laburnum* cells. Hence the seedlings should be, as they have proved themselves to be, pure *Laburnum* plants. Baur's hypothesis, therefore, solves most of the difficulties which have beset the interpretation of the various phenomena that have been observed in connection with this mysterious plant, and his suggestion has found general acceptance, except perhaps by Blaringhem (1923) who is still doubtful as to its chimaeric nature.

IV. CRATAEGOMESPILUS.

The third case on record of the production of what was regarded as a graft hybrid is that of the so-called Néflier de Bronvaux, now generally known as *Crataegomespilus*. This union of what are usually regarded as two distinct genera, the hawthorn (*Crataegus monogyna*) and the medlar (*Mespilus germanica*), was the result of the normal horticultural practice of grafting a scion of the medlar on a hawthorn stock. In Dardar's garden in Bronvaux, near Metz, it was noticed in a medlar tree estimated to be about 100 years old that, at the point of union of stock and scion, two branches arose which differed in character from both the medlar and the hawthorn. These branches were propagated by grafting and distributed to most botanical and many private gardens by the horticultural firm of Simon-Louis Frères, whose dendrological expert, Mr E. Jouin (1899), described the two forms in some

detail in *Le Jardin*. They have since been referred to in many horticultural journals, but the first scientific and critical examination of them was made by Noll (1905).

Of the two branches mentioned above, one had more similarity with the medlar and was at first called *Mespilus Dardari*, while the other which had a closer resemblance to the hawthorn was named *Mespilus Jules d'Asnière* or *M. Asnieresii*. A point of interest in connection with these two graft hybrids is the fact that, contrary to the inability to obtain a seed hybrid between *Laburnum vulgare* and *Cytisus purpureus*, several presumptive seed hybrids of hawthorn and medlar have been described. This may have influenced some botanists to include both in the genus *Mespilus*. Focke (1881) instances a wild hybrid of *Mespilus germanica* and *Crataegus monogyna* found near Autun in France, and a cultivated thorn variously designated as *Mespilus Smithii* DC., *M. grandiflora* Sm., *Crataegus grandiflora* Hort and *C. lobata* Bosc. which arose in a horticultural garden in Hammersmith about 1820, is also supposed to be a seed hybrid of the medlar and the hawthorn. Both these presumptive hybrids differ one from the other, and a comparison of them with the two graft hybrids mentioned above shows that the presumptive seed hybrids and the graft hybrids are essentially different as, indeed, Noll has pointed out. This is of some importance, as some botanists, like Laurent (1902), unwilling to accept the possibility of the existence of graft hybrids, have assumed that the stock upon which the Bronvaux medlar was grafted was not a pure hawthorn, but a hybrid plant. Apart, however, from the difference between the presumptive seed hybrids and the graft hybrids, we have the assurance given to Noll by Mr E. Louis of Metz that the stock had sent out several shoots which were undoubtedly true *Crataegus monogyna*.

Details of the differences between the graft hybrids and their parental components are given by Noll (1905) without figures, but a well-illustrated account was published later by Meyer (1915), and the flowering shoots are well reproduced by Bean (1911) in the Kew Bulletin.

Crataegomespilus Dardari has leaves very like the medlar in shape though somewhat smaller in size (see Fig. 1c). Instead of the single flower of the medlar it bears some three or more flowers slightly smaller than those of the medlar, and with only two or three in place of five styles. The fruits are smaller than those of the medlar, but of the same brown colour. It might, in fact, be a smaller form of the medlar, were it not for the more numerous flowers and slight modifications. That it is not a pure form, however, is shown by the fact that, like other graft hybrids, it occasionally reverts back to the true medlar and also at times to the *Jules d'Asnière* type of graft hybrid.

This latter form, *Crataegomespilus Asnieresii*, which, as stated above, is more like the hawthorn in appearance, differs from the latter by the hirsute character of its leaves and flowering shoots as well as of its receptacle, while in shape the leaves are either oval like those of the medlar but very much smaller, or are trilobed (see Fig. 1b). On the long shoots the leaves are much more hawthorn-like, but this applies also to the *Crataegomespilus Dardari*. The flowers are small and in clusters resembling those of the hawthorn, and with one to two styles. The small fruits resemble those of the hawthorn in size and shape, but are brown in colour like

those of the medlar. Their seeds are often viable and give rise to plants indistinguishable from hawthorns. Like *Crataegomespilus Dardari*, *Crataegomespilus Asnieresii* may give rise by bud variation to pure hawthorn and very occasionally to pure medlar branches.

Noll, who examined the internal structure, noted some differences between the graft hybrids and the component parents, but his account is not particularly helpful in this respect. His observations, however, led him to the conclusion that in the case of the *Crataegomespili* (Néfliers de Bronvaux) we had to do with true graft hybrids, and he assumes that there has been a conjugation of two vegetative nuclei in the callus region. He considered that, in this case, the nuclei of the graft hybrid would be tetraploid though, by some auto-regulatory process, they might have

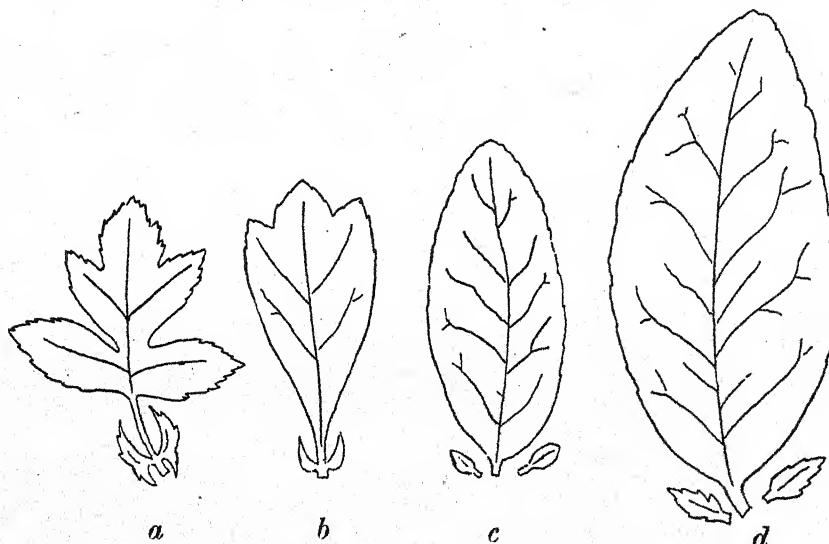


Fig. 1. Leaves of the *Crataegomespili* of Bronvaux and their parents. Natural size except *d.* which represents a small leaf of the medlar. *a.* Hawthorn. *b.* *Crataegomespilus Asnieresii.* *c.* *Crataegomespilus Dardari.* *d.* Medlar.

undergone a reduction division. He was unable, however, to get any cytological evidence.

Meyer (1915) gave a very full and well-illustrated account of the external and internal structure of the *Crataegomespili*, and was able by better cytological methods to determine the number of chromosomes in these hybrids and also in the parental plants. He found them in all cases to be 32 in the somatic cells. His investigation was, however, not undertaken like that of Noll with the object of detecting a possible fusion of vegetative cells, but since Baur had in the meantime put forward the view that the *Crataegomespili* were periclinal chimaeras, he was anxious to establish their periclinal structure from chromosome counts, which had elucidated in 1910 the constitution of the chimaeras obtained by Winkler in the case of his *Solanum* grafts. Though unable on a numerical basis to confirm Baur's hypothesis, Meyer found

that the individual chromosomes were shorter in the hawthorn than in the medlar, and he asserted that it was possible in *Crataegomespilus Asnieresii* to determine that the epidermal layer belonged to the *Mespilus* component, while the remaining tissues were formed from *Crataegus* cells. In the case of the form *Crataegomespilus Dardari* he was unable to determine the exact composition of the tissues. He accepts, however, from his detailed investigations, Baur's (1910) hypothesis that *Crataegomespilus Asnieresii* has a core of *Crataegus* covered by an epidermis of *Mespilus* (haplo-chlamydeous), and assumes that *Crataegomespilus Dardari*, which resembles the medlar more closely, has at least two layers of cells of the latter plant covering the core of hawthorn (diplochlamydeous).

The structure of the fruit of *Crataegomespilus Asnieresii* described and figured by Baur in his *Einführung in die experimentelle Vererbungslehre* (pl. IX) is perhaps the most striking illustration of its haplochlamydeous condition. The fruits of the graft hybrid are small in size, and not unlike those of the hawthorn in shape, but differ from them by their brown colour in which they resemble the fruits of the medlar. The red colour of the hawthorn fruits is due to anthocyanin contained particularly in the epidermal, but also in two or three adjacent layers of cells. The brown colour of the medlar is due to a brown pigment contained in the two outermost layers of cork cells, which are formed as in all Pomeae from the epidermis. In the fruits of *Crataegomespilus Asnieresii* in which the epidermis alone is supposed to be derived from the medlar, the epidermal layer has divided into several layers of cork cells, the outer of which are coloured brown, while the sub-epidermal cells contain the red-colour characteristic of the hawthorn berries. This particular appearance would, at first sight, be a remarkable support for the interpretation given to this graft hybrid by Baur and J. Meyer.

Noll and Baur have both germinated seeds of *Crataegomespilus Asnieresii*, which occasionally produces fertile seeds and the resulting seedlings have so far seemed to be pure hawthorns. This is in accord with the results obtained by the germination of the seeds of *Cytisus Adami* and, as was pointed out in connection with that plant, is in agreement with the view that the constitution of this *Crataegomespilus* is a haplochlamydeous periclinal chimaera. For, since the reproductive cells are produced from the sub-epidermal layer and only the epidermal layer of this chimaera is assumed to belong to the medlar, the reproductive cells should be pure hawthorn cells.

But in spite of the detailed examination of the *Crataegomespili* by Baur and Johannes Meyer, one very obvious feature of these plants was overlooked by them. The leaf tissues, like other parts of the plant, had been examined in transverse sections, as a periclinal arrangement of tissues was most likely to reveal itself, when the outer and inner tissues could be studied at the same time, but curiously enough the epidermal cells had not been examined in surface view, in which case a very different result is obtained. Judging from the sections of leaves figured by Meyer (Fig. 16, p. 24) one might imagine that the cells of *Mespilus* were narrower as well as deeper than those of *Crataegus*, while a surface view shows that they are much larger and not straight in outline but very wavy and, therefore, the small irregular

narrow portions which appear in transverse section are only bays in the cell outline and not individual cells. This appears from the photographic representation of the epidermal surface of the leaves given by Weiss (1925), and reproduced in Fig. 2. If one compares the epidermal cells of *Crataegus* and *Mespilus* with those of the two forms of graft hybrids, the result is striking. In both of these one ought, according to Baur's view, to have the same kind of epidermal cell as one finds in *Mespilus*. This is, however, not the case. The epidermal cells of *Crataegomespilus Asnieresii* are very much more like those of *Crataegus* both in size and shape, though there is an occasional indication of slight waviness. They would certainly be more likely to be mistaken for those of *Crataegus* than for those of *Mespilus*. Even the leaves of

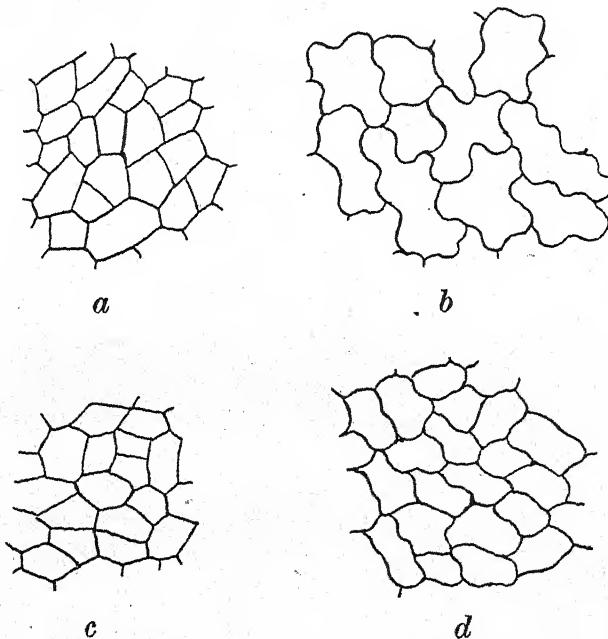


Fig. 2. Cells of the upper epidermis of the *Crataegomespili* of Bronvaux and their parents. $\times 250$.
a. Hawthorn. b. Medlar. c. *Crataegomespilus Asnieresii*. d. *Crataegomespilus Dardari*.

Crataegomespilus Dardari, which are very like those of *Mespilus* in their shape and appearance though somewhat smaller in size, have epidermal cells which have a good deal of resemblance to those of the hawthorn, though they show a certain waviness in their outline like those of *Mespilus*. They might well be regarded as intermediate in character, very much as one might have expected in the case of a seed hybrid. It is, therefore, not so easy to accept without reservation Baur's explanation of these graft hybrids as periclinal chimaeras in which the individuality of the component tissues remains unchanged. Weiss' observations were confirmed by Haberlandt (1926, 1927). In the very careful and critical examination of the tissues of the *Crataegomespili* of Bronvaux and of their parents, more particularly of the foliar organs, this leading plant anatomist has brought together a number of important

observations, some confirmatory of the observations of Meyer and Baur, and thus supporting the periclinal arrangement of the parental tissues, but others indicating a mixture of characters. In addition to the shape and size of the epidermal cells mentioned above, Haberlandt finds that the stomata of the *Crataegomespili* represent in many characters an intermediate condition between those of *Crataegus* and of *Mespilus*, and he also finds many instances of abnormal development of the stomata such as he has observed in many species hybrids. The vascular supply to the leaves of the graft hybrids also does not conform with the arrangement which would be characteristic of periclinal chimaeras, namely, with that of *Crataegus*, but while varying a good deal, particularly in *Crataegomespilus Dardari*, has a great deal of resemblance to that of *Mespilus*. Haberlandt therefore summarises his examination by the statement that in the anatomical structure of the leaves of the *Crataegomespili* of Bronvaux, the histological characters represent conditions partly intermediate between the two parents *Mespilus germanica* and *Crataegus monogyna* and partly mosaic-like combinations of the parental characters.

He comes to the conclusion, therefore, that they do not represent, as suggested by E. Baur and J. Meyer, periclinal chimaeras with a *pure Crataegus* core and a *pure Mespilus* epidermis, though he is not prepared to accept the alternative view put forward by Noll that they represent hybrids formed by fusion of vegetative cells of the stock and the scion, a condition which has been termed a "burdo." Some features, it is true, like those referred to above, might point in that direction, but there are others which it would be more difficult to harmonise with the conception of a vegetative hybrid. Thus two so strikingly different forms, *C. Dardari* and *C. Asnieresii*, are not usual even as reciprocal crosses and could only be explained as Noll had done by assuming that in one case the nucleus of a *Crataegus* cell had entered into a *Mespilus* cell and that in the other hybrid the reverse process had taken place. Haberlandt considers this to be an unlikely explanation, and thinks that it would be easier to suppose that in the reduction division which must have taken place after fusion, since the hybrids have the same number of chromosomes as the parents, there may have been some irregularity in the division.

Another difficulty in accepting the "burdo" theory presents itself in the frequent reversion of the graft hybrid to the parental forms, a feature characteristic, as we have seen, of other graft hybrids. It is, of course, known to take place, but much less frequently, in seed hybrids. But, though this reversion is more characteristic of graft hybrids and can readily be explained by the chimaera hypothesis, it is possible that in fusion nuclei formed in a vegetative way, the parental characters may more easily segregate than is the case with nuclei formed by a fusion of generative cells. The reduced fertility of *Crataegomespilus Asnieresii* and the sterility of *Crataegomespilus Dardari* are conditions frequent in seed hybrids and might also be found in a graft hybrid. The production of apparently pure *Crataegus* seedlings from the fertile seeds of *Crataegomespilus Asnieresii*, on the other hand, seems a strong argument in favour of the periclinal chimaera theory. But, as Haberlandt points out, we have no information whether the ovules had been fertilised by *Crataegus* pollen or by that of *Crataegomespilus* itself.

The supporters of the periclinal chimaera theory may argue that the protoplasmic connection which has been shown by Strasburger to exist between the cells of stock and scion, by Buder (1911) between the epidermal and sub-epidermal cells of *Cytisus Adami*, and by Hume (1913) and Meyer (1914) in other chimaeras, probably also exists between the component parts of the *Crataegomespili*, and may have a modifying effect, so that in the case of the *Crataegomespili* the epidermal cells of the medlar may have taken over some of the characters of the *Crataegus* stock. But the very fundamental difference in the case of the epidermal cell of *Crataegomespilus Asnieresii* could hardly be explained in this way, nor could the deep-seated changes in the leaf stalks of the hybrids be attributed to the epidermal layer of *Mespilus*. In the ovary of *Crataegomespilus Dardari*, Weiss (1925) found not infrequently two or even three loculi and correspondingly two styles, a morphological change in the structure of the flower which could hardly be due to a covering layer of *Mespilus* cells.

After carefully reviewing all the characters which seem to support the chimaera theory and all those which are incompatible with it, and which seem to point to a true vegetative hybrid nature of the *Crataegomespili*, Haberlandt leaves the matter for the present an open question. On the other hand, he rejects, like E. Baur, the view that these curious results of grafting are of the nature of hyperchimaeras in the sense of Strasburger.

The chimaera theory of graft hybrids derives some support from the occurrence of two forms in the case of *Crataegomespilus*, for one would not expect this in the case of seed hybrids in which usually only one hybrid form occurs even from reciprocal crossings. In hyperchimaeras, such as Strasburger envisages, the number of possible graft hybrids might be very numerous; in the case of periclinal chimaeras, at least four possibilities occur, all four of which have been realised experimentally by Winkler in the case of *Solanum*, as will be seen later on. Of *Crataegomespilus* several other forms occur besides those known as *C. Asnieresii* and *C. Dardari* and described above. Noll in 1905 describes a third form as having originated on the *Mespilus* of Bronvaux on the opposite side of the stem from the two mentioned above, which later became very similar to the *Asnieresii* branch, but differed from it by being completely sterile. For this form he suggested the name of *Crataegomespilus Jouini*.

Lucien Daniel (1914) has described two further *Crataegomespili* of different origin from those of Bronvaux, that may be referred to as the *Crataegomespili* of Saujon, the locality in which they arose. Daniel's attention was drawn to them in 1906 by Captain Brun, who had been experimenting in obtaining graft hybrids. In the case of these hybrids a young hawthorn had been allowed to grow 1½ metres in height, and on five of its branches scions of the medlar had been inserted. All the grafts were successful and for 15 years duly produced normal medlars. Then when one of the branches began to show signs of decrepitude some adventitious buds appeared at the seat of grafting. Two years later the branch was snapped off by the wind and the two adventitious buds developed further. One of these, according to Daniel, developed into a pure medlar, but of the wild type with thorns, while the

other gave rise to several shoots, one being a pure hawthorn identical with branches found at the base of the stem, two of the others showed intermediate characters and were named by him *Crataegomespilus Bonnierii* and *C. Bruni* respectively. The former had medlar-like leaves, but of smaller size, and numerous flowers arranged in a corymb. The flowers and fruits were rather like those of *Crataegomespilus Asnieresii*, the fruits being brown in colour and containing apparently mature seeds, which did not, however, germinate. This form, therefore, stands somewhat midway between *Crataegomespilus Dardari* and *C. Asnieresii*. The second form from Saujon, *Crataegomespilus Bruni*, has the divided leaf covered with hairs characteristic of *Crataegomespilus Asnieresii*. The fruits are variable in colour, sometimes entirely brown, sometimes sectorially coloured red and brown. The calyx may, according to Daniel, drop away when the fruit matures as it does in the hawthorn, or may persist. The figures given by Daniel, however, are not convincing on this point, as he seems to have figured a reversion to the hawthorn in Fig. 3, judging by the foliage as well as the fruits. The special feature, however, seems to be the weeping habit of the branch, which is perhaps to be looked upon as a bud variation independent of the formation of the graft hybrid. This weeping habit showed itself also in the branches which later reverted to the hawthorn, as reported by Daniel in 1919. To this weeping variety of hawthorn, Daniel gives the name of *Crataegomespilus Rivierii*. It seems questionable, however, whether it contains any *Mespilus* element.

The two *Crataegomespili* from Saujon Daniel has examined anatomically, and his observations go far to show that, as in the *Crataegomespili* from Bronvaux, we are not dealing with a pure core of hawthorn surrounded by pure epidermis of the medlar, but that we have more of a blending of characters. The ripe fruit of the medlar, unlike that of the hawthorn, contains numerous stone cells some eight or ten cells below the epidermis. Such stone cells occur also in the fleshy portion of the fruit of *Crataegomespilus Bonnierii* in tissues which, according to Baur's hypothesis, should belong to the *Crataegus* core and should, therefore, be without them. Similarly deep in the tissues of the sepals of *Crataegomespilus Bonnierii* we find sclerenchymatous cells which do not occur in the hawthorn, but are characteristic of the sepals of the medlar. Daniel further draws attention to a new feature in this *Crataegomespilus* which does not occur in either of the parents, namely, the existence of three rows of palisade cells in both sides of the sepals, which in this form are not spread out, but arch over the apex of the fruit. Apart, however, from this new character upon which Daniel lays some stress, as it bears out his view that new characters may arise by what he calls "hybridation asexuelle," the *Crataegomespili* of Saujon bear out the results obtained by Weiss and Haberlandt in connection with the *Crataegomespili* of Bronvaux, and make it impossible to accept without reservation the interpretation of these graft hybrids as pure periclinal chimaeras.

Through the kindness of Prof. Daniel, I have been able to examine the epidermal cells of the two *Crataegomespili* from Saujon. They bear out the observations previously made (1925) on the *Crataegomespili* of Bronvaux. The epidermal cells of *Crataegomespilus Bruni* are small and more like those of the hawthorn than the medlar. They appear, however, somewhat variable, and a few of them show a

tendency to have slightly wavy walls, though nothing like those of the medlar. *Crataegomespilus Bonnieri* has somewhat larger epidermal cells somewhat intermediate in shape between those of the hawthorn and the medlar, much more like what one would expect in a true hybrid than in a chimaera with a pure medlar epidermis.

Two other less well-known and less carefully examined *Crataegomespili* have been described. The first was observed by Manaresi (1915), whose published account I have unfortunately not been able to see. Probably this difficulty may have been experienced by others, as little information is available concerning it. The form more recently described by Seeliger (1926) was discovered in 1913 by Ludwig Lange, also in the neighbourhood of Metz, and has been called *Crataegomespilus Langei*. In this case the stock upon which the medlar was grafted was not *Crataegus monogyna*, but *Crataegus oxyacantha*. As only dried material was examined nothing is known as to reversion to its components. The leaves appear rather like those of *Crataegomespilus Asnieresii*, but Lange thinks it is most like *Crataegomespilus Bruni*, the smaller form of the Saujon material. Lange figures the epidermis which agrees with that figured by Weiss and Haberlandt for *Crataegomespilus Asnieresii*, that is, the epidermal cells are more like those of the hawthorn than the medlar. Like the other *Crataegomespili*, Seeliger's specimen is more hirsute than the hawthorn but, while the *Asnieresii* and *Dardari* forms have approximately the same number of hairs as the medlar has, *Crataegomespilus Langei* has a great many more than the medlar, the numbers given by Seelinger are per 10 square millimetres:

		Upper surface	Under surface
<i>Crataegus</i>	...	6-7	3-4
<i>Crataegomespilus Langei</i>		116	354
<i>Mespilus</i>	...	63	251

Daniel would probably regard this form as "un hybride de greffe renforcé."

V. SOME NEW OR PROBLEMATICAL GRAFT HYBRIDS.

A number of other cases of graft hybrids have been described, some of which we know the origin, while in the case of others there is some doubt as to their inclusion among the category of hybrids or chimaeras resulting from grafting.

Populus. Baur reports in his *Einführung in die experimentelle Vererbungslehre* that both sectorial and periclinal chimaeras can be readily obtained by budding *Populus nigra* on *Populus trichocarpa* and *vice versa* in August, and cutting across the union of the two in the next July, when the shoot has developed from the inserted bud.

Piroydonia. It is a common practice in horticulture, as has been shown by Hatton (1920), to graft the pear (*Pyrus communis*) on a quince (*Cydonia vulgaris*) stock, as it is considered that the pear fruits at an earlier age when thus grafted. Often, when the tree is old, signs of decrepitude show themselves and the trees are then cut down to the point of the original grafting, after which one or more of the branches formed from adventitious buds is encouraged to grow into a fruiting branch. Such a procedure was adopted in the garden of St Vincent at Rennes in

1902. On one of these trees two shoots arising below the graft were pure shoots of the quince, while three which arose at the point of union of stock and scion showed intermediate characters which were examined and described by Lucien Daniel (1904). The name of *Pirocydonia Danieli* has been given by Hans Winkler to this graft hybrid.

The leaves, according to Daniel's description, are less hairy than the leaves of the quince, but more hairy than those of the pear and the hairs tend to persist for a longer period than do those of the pear, more particularly those on the under-surface of the leaf. In shape the leaves of *Pirocydonia* resemble those of the quince in being broader and rounder, particularly at the base, while they taper away at their apex, more like those of the pear. The most peculiar character, however, is the difference in the margin of the leaf. In the quince the leaf margin is entire, in the pear it is serrate. In *Pirocydonia* the broader and more rounded base is entire, while the

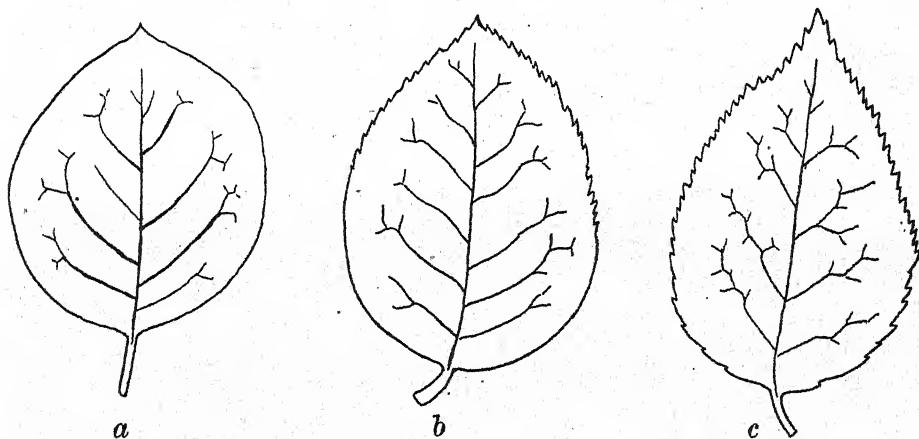


Fig. 3. The leaves of *Pirocydonia Danieli* and its parents, drawn natural size. a. Quince (*Cydonia vulgaris*). b. *Pirocydonia Danieli*. c. Pear (*Pyrus communis*). Note the differences of the leaf-margins.

narrower, more pear-like upper portion of the leaf is serrate. The leaf, indeed, appears to be made up of a quince-like basal and a pear-like apical portion. Such an arrangement is entirely different from any other mixture of characters met with in graft hybrids, and cannot be explained by a periclinal or sectorial arrangement of tissues. In 1922 when I visited Rennes to inspect for myself some of Prof. Daniel's interesting plants, I was enabled through his kindness to obtain some material of the leaves of *Pirocydonia*, from which the accompanying drawings have been made (Fig. 3).

Daniel has examined and described the anatomy of the leaves of *Pirocydonia* and its parents, and from his figures and description it would appear that the internal structure of the leaves of the graft hybrid also presents features intermediate between those of the quince and the pear. He does not, however, indicate whether there is any structural difference between the upper and the lower halves of the leaves as might be expected.

From the material at my disposal, through the kindness of Prof. Daniel, I am able to add the following. The epidermal cells of the quince are smaller than those of the pear and have only a slightly wavy outline when examined in surface view, while the larger cells of the pear have a very wavy outline very much like the epidermal cells of the medlar. The epidermal cells of *Pirocydonia* when seen in surface have a variable but distinctly intermediate size and shape. Nor can any distinction be seen between the cells of the lower half of the leaf and the upper half, in spite of the fact that the former is more quince-like and the latter more pear-like. From Daniel's observations and my own, I think there is every reason to regard *Pirocydonia* as a true graft hybrid. Unfortunately, so far it has produced no flowers, though cultivated for 20 years as a graft in the ordinary way. Nor had it when I saw it in 1923 produced any reverions to either of the parental components; in this it differs from *Cytisus Adami* and other chimaeras.

Daniel (1913) was able to make further experiments in the production of graft hybrids by having at his disposal, in a deserted garden, a number of old pear trees which he decapitated down to the point of union of stock and scion. From this point (bourrelet) numerous adventitious buds arose, some of pure quince, others intermediate in character to the quince and pear, and similar to the *Pirocydonia Danieli* obtained in 1902.

In addition to this form Daniel reports the occurrence of another form more fully described by D. Bois (1914), and to which the name of *Pirocydonia Winkleri* was given. This shoot arose as a sucker from the root of the quince some 5 or 6 cm. below the graft, and produced a growth of short and bushy stature with leaves differing in several respects from those of the quince. Daniel describes them as more pubescent than those of the quince, more lanceolate and narrowing towards the apex, like the leaves of the pear. It is certainly different in its general habit and aspect from the common quince.

At the International Horticultural Congress in Amsterdam 1923, when Prof. Daniel gave an account of this plant, Bateson suggested that it might be a "sport" from the quince root and this seems to me the more probable explanation for two reasons. In the first place its leaves do not show any trace of the teeth characteristic of the pear, which are a definite character of *Pirocydonia Danieli*. In the second place the behaviour of this form to the attacks of the fungus *Aecidium cancellatum* is of interest. This fungus attacks the quince, but not the pear. In the case of *Pirocydonia Danieli* the fungus attacks the leaves and causes slight discoloured patches, but never produces aecidia, the quince element evidently rendering the leaves resistant to further progress of the fungus. *Pirocydonia Winkleri* behaves like *Cydonia vulgaris*, and is not attacked at all.

Pirocrataegus Willei. This apparent graft hybrid formed by a graft of the pear on the hawthorn stock was described by Wille in 1896. The tree was a pear grafted on to a hawthorn stock, and was found growing at Borge in Norway. The leaves were not intermediate in character, but like those of the pear. The tree did not flower for about 15 years, after which it produced pear-like flowers, but of a small size and in crowded corymbs like those of the hawthorn. The fruits were pear-shaped, but

small and of the red colour of the hawthorn fruits. The fruit had five loculi like the pear, but the seeds were sterile. There was no reversion of branches to the hawthorn, though shoots of the latter sprouted from below the graft. The ordinary characteristics of graft hybrids did not show themselves.

Prof. Wille was good enough to send me some leaves of this plant in 1916. Flowers were not available that year, as it rarely flowers. In his accompanying letter Prof. Wille said: "I now think it is rather doubtful whether it really is a graft hybrid." Holmboe (1905) had suggested that it was probably *Pyrus polloeria*.

Amygdalopersica. The appearance of branches bearing flowers and apparently fruits of the almond on a peach tree grafted on an almond stock was chronicled by Mr Formont, a fact which Daniel (1913) believes to indicate the transmission of the characters of the almond stock to the grafted peach. It may, however, be a case of somatic segregation of a hybrid and consequently heterozygous peach. The probability of this explanation is increased by the fact that the same feature appeared in two plants of the same nursery, where probably the scions from the same peach were used. In 1905, at Mas-Grenier in the Tarn-et-Garonne department of France, an almond tree was cut down to a short distance from its base (collet). It developed a number of adventitious shoots, on which were inserted in August a number of resting buds of a particular variety of peach with yellow fruits usually cultivated in that district, and which up to then had shown no sort of variation. The buds developed normally, but in the second year branches commenced to change in a singular manner and produced fruits which were intermediate between the peach and the almond. Their flesh was thin and yellow and then shrivelled up into a green covering of the shell, similar to that of an almond. The stones or shells were intermediate in character between those of the peach and the almond with sculpturing of the stone less deep than the peach, but obviously different from the shell of the almond. The grafted branches bore fruiting spurs with pure almonds, others with peach and others with the intermediate products.

Amygdalopersica differs markedly in several respects from the other known graft hybrids. It is obviously not a periclinal arrangement of tissues, as the stone is affected by it. As Daniel also points out the branches which show the instances of reversion to the almond and intermediate fruits do not arise at the junction of stock and scion, but at some distance from this region. Believing as he does in the transmission of characters from stock to scion, he regards these as asexual hybrids developed by a sort of growth *par entraînement*. The *Amygdalopersica* he describes must, I think, still be classed among problematical graft hybrids. Possibly this case may be referred to somatic segregation of a heterozygous slip which may have been inserted on the almond stock. A form known as *Prunus persica-aamygdalo* Duh. is regarded by Schneider (1906) as probably a hybrid of *Prunus Amygdalus* × *persica*, and is said to produce dry and shrivelled fruits with stones intermediate between those of a peach and an almond, on the whole more like the former. It might be that a slip of this plant had been grafted in place of a peach. This explanation would not, however, explain the fact that the graft commenced by producing normal yellow peaches.

Of some of the other chimaeras which have been described, that called a *Peach-Nectarine*, by Ikeno and Noguchi (1929), seems most probably to be a case of somatic segregation of a heterozygous plant, since peach and nectarine can be readily crossed, and though usually the skin is intermediate as regards hirsuteness, occasionally smoothness is dominant. It seems uncertain in this case whether the tree is a peach or a nectarine. A point of interest in connection with this chimaera is the fact that segregation is not always sectorial in the ordinary sense of a longitudinal diversity of tissues, but sometimes, in part at all events, transverse, a fact which makes a comparison with the leaf of *Pirocydonia* possible, though in this latter case there is not abrupt transition as in the *Peach-Nectarine* chimaera figured by Ikeno.

Olive. The graft hybrids of the olive, described by La Marca (1918), seem really to be a case of reversion by bud variation of a cultivated white-fruited variety of heterozygous constitution, to a probably dark-fruited ancestor.

Rosa. Graft hybrids of roses have been described by Caspary (1865) and by Daniel (1924), but neither of them throws much light on the nature of such hybrids nor of chimaeras, nor do the various cases of graft hybrids or chimaeras mentioned in the horticultural journals by Dammer (1912) in *Robinia*, and by Loebner (1913) in *Rhododendron*.

VI. SOLANUM.

While the discussions regarding the nature of the supposed graft hybrids of *Citrus* (*bizzarria*), *Cytisus Adami* and of *Crataegomespilus* were in progress at the beginning of the nineteenth century, and efforts to obtain them again by grafting had been unsuccessful, Hans Winkler in Germany set out to study the problem in the only satisfactory manner, namely, by experimental methods. Choosing for that purpose plants which could be easily grafted and which were prone to produce adventitious buds, as well as exhibiting sufficiently distinct specific characters, Winkler's work proved to be highly successful. From Voechting's experience in transplantation of vegetable tissues, Winkler (1907) chose herbaceous rather than woody plants, and conducted his experiments by grafting young shoots of the tomato (*Solanum lycopersicum*) on the stem of the nightshade (*Solanum nigrum*) and *vice versa*. His method of procedure was to unite, by wedge or saddle grafting, the young stems of the tomato with those of the nightshade. The grafting proved to be easily effected. When the union of the two plants had taken place, a horizontal cut was made at the point of union, and thus the united tissues of both scion and stock were exposed. The wound was quickly healed over by the development of callus and adventitious buds were formed, giving rise sometimes to pure tomato, sometimes to pure nightshade shoots, according to the position in which they were formed. Occasionally if the buds arose actually at the juncture of the tissues of the two species, the bud produced a shoot bearing the leaves of the tomato on one side and those of the nightshade on the other. Such a sectorial shoot is figured by Winkler (1907, p. 573) in his first paper on the subject. In spite of the very different nature of the two kinds of tissues, the shoot grew quite normally

and straight; and Winkler suggested the name of "chimaera" for it, after the fabulous mythological monster, part lion part dragon. This sectorial chimaera was exhibited by Winkler at the meeting of German naturalists at Dresden in 1907. In the following year he obtained another and more striking growth from a similar graft, in which a shoot of the tomato had been inserted in the nightshade stock. In this case the shoot did not exhibit the specific characters of the two united individual plants as in the case of the earlier chimaera, but bore leaves which were intermediate in character, being undivided like those of *Solanum nigrum*, but having the serrate margin and the hairy covering of the tomato. This growth Winkler regarded as a true graft hybrid, and named it *Solanum tubingense*. The plant produced flowers which were also of intermediate character in size and colour, and ultimately fruits which were black like those of *Solanum nigrum*, but slightly larger.

A year later Winkler (1909) was able to record cases of reversion of certain branches of *Solanum tubingense* to *Solanum nigrum*, thus bringing his newly created graft hybrid into line, as far as its behaviour was concerned, with that characteristic of the other presumptive graft hybrids, the bizzarria orange, *Cytisus Adami* and the *Crataegomespili*.

In the meantime, Erwin Baur (1909) and Vestergren (1909), in reviewing Winkler's work, had expressed doubts as to the true hybrid nature of *Solanum tubingense*, that is, as to an actual fusion of vegetative cells having taken place, and Baur, basing his opinion on the observations and experiments he had made in the production of white-margined varieties of *Pelargonium* (vars. *albomarginatae hort.*), suggested that *Solanum tubingense* might prove to be a periclinal chimaera. Strasburger (1909) in a similar critical article, in which he defines his position towards the question of graft hybrids generally, and after having been unable to discover indications of nuclear fusion or binucleate cells in graftings of *Solanum nigrum* and *Solanum lycopersicum*, expressed some doubt as to the true hybrid nature of *Solanum tubingense*. But, owing to its obvious difference in structure from a sectorial chimaera and to the apparently intermediate character of its organs, he suggests that here, as in other cases of presumptive graft hybrids, there may be a very considerable and intimate mixture of component cells derived from the two parental plants, and that in the case of such an interpenetration of tissues, there is a reciprocal influence upon one another of the characters of the two species. He considered that the specific activities of the chromosomes in the nuclei of both species which were united in so intimate a way might exert a similar influence upon the development as if the chromosomes were united in a sexual hybrid in the same nucleus. To this climax of chimaeric formation in which an interpenetration of tissues takes the place of nuclear fusion, Strasburger gave the name of "hyper-chimaera."

Though Winkler was not inclined to accept either Baur's or Strasburger's interpretation of the nature of *Solanum tubingense*, he continued his fruitful experiments and soon produced a varied series of new growths. Several of these were described in the *Zeitschrift für Botanik* in 1909 and include the very variable form *Solanum proteus*, and the more constant *Solanum Darwinianum*, both obtained by

grafting shoots of the tomato on the nightshade as stock. Two other forms *Solanum Koelreuterianum* and *Solanum Gaertnerianum* were obtained by the reciprocal grafting, namely from shoots of the nightshade inserted on the stock of the tomato. The characters of these different forms are now familiar to students by the figures in Strasburger's textbook. In view of this variety of forms it was difficult to maintain that a fusion of nuclei of the two plants had taken place in all five cases, as one would only expect one or at most two different reciprocal forms by nuclear fusion. The chimaera hypothesis was, therefore, in the ascendant as an explanation of these new formations. Luckily a further criterion was available. Unlike the other cases of graft hybrids, the constituent plants in the case of the Solanums chosen by Winkler differed materially in the number of their chromosomes. In *Solanum lycopersicum* the haploid number is 12 while in *Solanum nigrum* it is 36, a diversity of numbers which may possibly account for the fact that the two species do not produce a seed hybrid. According to Winkler's determination, with the exception of *Solanum Darwinianum*, the graft hybrids had, in their reproductive cells, not the number 48 which might have been expected of them had there been a fusion of vegetative cells, nor the number 24 which might have occurred had the vegetative fusion been followed by some auto-regulatory reduction in the number of chromosomes, as might take place according to Nemec's experimental work on nuclei. The reproductive cells contained either 12 or 36 chromosomes; they agreed, therefore with either one or the other of the parental components. *Solanum tubingense* and *S. Gaertnerianum* showed 12 while *Solanum proteus* and *S. Koelreuterianum* possessed 36 chromosomes in their reproductive cells. The results of self-fertilisation of the various presumptive graft hybrids Winkler had obtained in 1910 and he was able to report upon them. In view of the chromosome counts just referred to, they are of particular interest, for they proved that these forms when fertile always give rise to pure descendants, either tomato plants or nightshade and, according to Winkler, to the parent to which the respective form is most nearly related by its general characters. Thus, of nearly 1200 seedlings raised by selfing *Solanum tubingense*, all were pure *Solanum nigrum*, as one would expect from the number of chromosomes of its reproductive cells. *Solanum Gaertnerianum*, which is less fertile, also produces nightshade plants, while *Solanum proteus* and *S. Koelreuterianum*, the reproductive cells of which contain only 12 chromosomes, produce tomato plants, which have the same number of chromosomes. This complete reversion of the seedlings to one of their parents is, as was pointed out in connection with *Cytisus Adami* and *Crataegomespilus Asnieresii*, the usual condition in graft hybrids. There is, therefore, no reason to regard Winkler's productions as differing in their nature from most of the so-called graft hybrids. They show the same somatic segregation of parental characters and also the seed reversion. The latter is adequately explained by Baur's view of a periclinal constitution of these chimaeras. The reproductive cells are normally formed in the Angiosperms from the sub-epidermal row of cells. If the periclinal chimaera is composed of the core of one parent covered only by the epidermis of the other, the reproductive cells would partake of the nature of the core. This is the case in *Solanum tubingense* and

S. Koelreuterianum, with the difference that the core in the former is *Solanum nigrum* and in the latter *Solanum lycopersicum*. Hence they produce different offspring. In the two more variable forms of *Solanum proteus* and *Solanum Gaertnerianum* we may suppose that the core of one of the parents is covered by a skin of the other, consisting of two layers of cells. This may account for the greater variability of their vegetative organs and it certainly determines the nature of the reproductive cells which, in these two cases, would partake of the nature of the skin parent. And this has been shown to be the case both by cytological examination and by self-fertilisation.

Supposing that the four graft hybrids mentioned above have the periclinal constitution just indicated, and Winkler now adopts this explanation of their nature, there remains something to be said about *Solanum Darwinianum* which does not fit into the periclinal chimaera hypothesis. In the first place, its reproductive cells have neither the number of chromosomes of those of the tomato nor of those of the nightshade, but 24, the diploid number of the tomato. Winkler assumes that *Solanum Darwinianum* had arisen as a "burdo," i.e. the result of the fusion of two vegetative cells of the tomato and the nightshade. As the former plant has 24 and the latter 72 chromosomes in the somatic nucleus, the "burdo" should have 96, and Winkler then assumes that an auto-regulatory reduction to 48 has taken place in the somatic cells. The reproductive cells should, therefore, after reduction have 24 chromosomes, the number actually found by Winkler, while the somatic cells ought to contain 48. It would appear, therefore, that we have here a case of fusion of vegetative cells to form a true graft hybrid as distinct from a chimaera; an argument in favour of the true hybrid nature is that this form of graft hybrid proved to be sterile. Baur (1919) does not consider this hybrid nature is proved unless it is known that all the vegetative tissues of *Solanum Darwinianum* have 48 chromosomes in their nuclei. This has apparently not yet been established. It is, however, impossible to do so now as this particular plant has died.

Another explanation of the nature of *Solanum Darwinianum* has been suggested by Winkler himself. He had shown (1916) that it is possible in making his grafts of *Solanum* to get in the wound tissue a fusion of nuclei of the same plants either of *Solanum nigrum* or of *Solanum lycopersicum* and thus to obtain tetraploid forms of these plants. Indeed, in some cases, he obtained a tetraploid core of one surrounded by the normal diploid skin of the other component. It is just possible, therefore, that *Solanum Darwinianum* had a tetraploid core of the tomato covered by an epidermis of the nightshade and, therefore, 24 instead of the 12 chromosomes in its reproductive cells. But Winkler does not consider this likely, though he did obtain one specimen of *Solanum Koelreuterianum* with a tetraploid core.

The possibility of the existence of diplochlamydeous periclinal chimaeras having been called into question by Noack (1922), as indicated further on, Maria Mayer-Alberti (1924) undertook under Winkler's direction a detailed and comparative examination of the foliage leaves of three of the graft hybrids, *Solanum tubingense*, *proteus* and *Koelreuterianum*, as well as of the component parents, with a view to ascertaining what portion of the parental cells took part in the formation of the

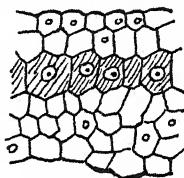
mature leaves. This examination confirmed the periclinal composition of the hybrids, and showed that in *Solanum proteus* the whole of the mesophyll as well as the epidermis was derived by division of the sub-epidermal layer from the tomato, and that only the vascular bundle and probably its sheath was formed from the tissues of the nightshade. Since this is the case and yet the leaf is so unlike that of the tomato, it is probable that the venation determines largely the shape of the leaf, and whether it is to be undivided or divided. All three hybrids have a serrate margin which can obviously not be determined merely by the epidermal layer, but points to an influencing of one set of tissues by the other. The shape of the epidermal cells, however, seems hardly influenced by the underlying tissue, for Mayer-Alberti's figures show that *Solanum tubingense* has epidermal cells of the same shape as the tomato, in spite of the underlying hawthorn core, while those of *Solanum Koelreuterianum* are identical in shape, though somewhat smaller in size, than those of the nightshade. In *Solanum proteus* in which, according to Mayer-Alberti, the whole of the mesophyll is composed of the tissues of tomato, the epidermal cells are somewhat intermediate in shape, as has been indicated above in the case for *Crataegomespilus Dardari*, where the skin tissue in the leaf is not less than two and probably more layers in thickness. A later and much more detailed account of the development and structure of the leaves of the *Solanum* chimaeras has been given by Lange (1927) as he undertook to corroborate or to refute the views of Noack that periclinal chimaeras with more than one layer of skin tissue, i.e. diplochlamydeous forms, could not exist. For Noack (1922) had shown that in the development of the leaves only the epidermal and the sub-epidermal tissues took part, the latter dividing by periclinal walls to form all the tissues of the leaf with the exception of the epidermis. Though Noack's investigations were mainly made upon *Pelargonium* with a view to combating Baur's hypothesis that the white-margined varieties were of the nature of diplochlamydeous periclinal chimaeras, he extended his investigations to *Solanum proteus* and *Crataegomespilus Dardari* which were also placed by Baur in the category of diplochlamydeous periclinal chimaeras. He came to the conclusion that not only in *Pelargonium*, but also in *Crataegomespilus Dardari* and in *Solanum proteus*, the whole leaf is developed from the dermatogen and the second layer of cells of the apex, the sub-epidermal layer dividing tangentially to form the whole of the leaf tissue, with the exception of the epidermis. There would be no possibility, therefore, of a diplochlamydeous periclinal chimaera, the whole leaf arising from two initial layers, and in the case of a diplochlamydeous covering of the stem apex, the leaves would be of one kind of tissue only. As far as *Solanum proteus* is concerned, however, Lange found that a certain portion of the mesophyll of the leaf, particularly of the central portion of it, is formed from the third layer of initial cells of the meristem, that is, from the *Solanum nigrum* core. This is in addition to the vascular bundles which, of course, are formed from the core. The leaf, therefore, partakes of the tissues of both parental components, as can easily be seen even in very young leaves by the size of the cells and also by that of the nuclei. The core tissue of *Solanum nigrum* thins out towards the edge of the leaf, where it is only one layer in thickness (see Fig. 4). In *Solanum Gaertnerianum*, the reciprocal diplochlamydeous

form, the core takes a somewhat slighter part in the formation of the mesophyll, and does not reach the margin, having an irregular shape well figured by Lange. His observations, therefore, contradict those of Noack and, since they are in agreement with those of Krumbholz (1925), the diplochlamydeous character of the leaves of *Solanum Gaertnerianum* and *S. proteus* may be considered to be established.

More recently Jörgensen and Crane (1927), adopting Winkler's methods, have produced a further series of *Solanum* chimaeras by experimenting with other species of this genus. Working at the John Innes Horticultural Institution, they



a



b

Fig. 4.

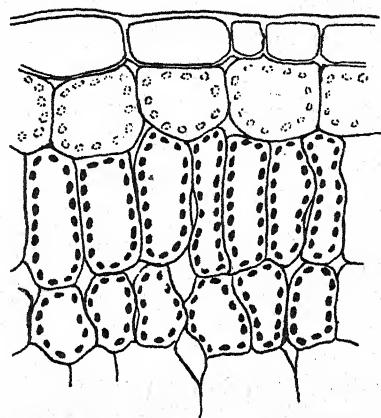


Fig. 5.

Fig. 4. Leaf structure of two diplochlamydeous *Solanum* chimaeras, after Schwarz. a. Diagrammatic representation of one half of the leaf of *Solanum Gaertnerianum*. The dotted line indicates the outer limit of the tissues of inner component of the chimaera (tomato). b. Transverse section of young leaf of *Solanum proteus* showing two layers of tomato skin on upper surface, three layers on lower side of leaf. The large innermost cells represent the nightshade constituent. These cells have larger nuclei with 72 chromosomes compared with 24 in the tomato cells.

Fig. 5. Portion of section of leaf of a white-margined *Pelargonium* after Baur, showing the sub-epidermal layer with colourless plastids and green plastids in the deeper cells of the mesophyll. Epidermis and sub-epidermis belong to a colourless parent.

have obtained the following forms, in designating which the first specific name indicates the nature of the core and the second that of the skin:

<i>Solanum nigrum</i> var. <i>gracile sisymbriifolium</i>	haplochlamydeous
<i>Solanum lycopersicum-guineense</i>	diplochlamydeous
<i>Solanum lycopersicum-luteum</i>	haplochlamydeous
<i>Solanum lycopersicum-luteum</i>	diplochlamydeous
<i>Solanum luteum-lycopersicum</i>	haplochlamydeous

These showed various tendencies to somatic segregation into the parental forms. Most *Solanum* species, owing to their tendency to form adventitious buds, are suitable for experimental work in this direction. *Solanum tuberosum*, the potato, on the other hand, shows more reluctance to the formation of adventitious buds, which may possibly be connected with the facility it has for tuber formation. Jörgensen (1927-1928) experimenting with this species and the tomato was able

to produce a periclinal chimaera, *Solanum lycopersicum-tuberosum*, in which the core of the tomato was covered in by an epidermal layer of the potato, but the reciprocal chimaera which he particularly desired he was unable to obtain.

Darwin (1888) in his discussion of graft hybrids gives a record of many instances of the production of parti-coloured tubers from the grafting of eyes of red potatoes on white tubers and *vice versa*, and also by uniting the stems of white and red varieties. Only a few of these experiments like those of Hildebrand (1869) and Magnus (1872) were conducted with scientific insight, the others did not lead to any clear understanding of the problems involved, and experiments on similar lines, now that we have a better knowledge of the probable constitution of graft hybrids, would be worth undertaking. In the meantime our knowledge of this economically important plant has been furthered by Asseyeva (1927). Examining a number of bud mutations in the potato, he has come to the conclusion that many of them are of a chimaeric nature. This he considers to be proved by two features which they exhibit:

- (1) When the eyes were removed from the tubers of the mutant the new buds formed from deeper layers were like the original form before mutation.
- (2) The characters of the mutant were not transmitted by seed, which would imply that the mutant was a haplochlamydeous chimaera.

It is, of course, of interest to know that a mutation may arise in the epidermal tissue of a plant, forming thus, to use Frost's terminology, "an autogenous chimaera" as contrasted with the "synthetic chimaeras" which have been so largely the subject of investigation in the genus *Solanum*.

VII. *PELARGONIUM* (vars. *ALBOMARGINATAE*).

As previously stated Baur's fruitful conception that graft hybrids were of the nature of periclinal chimaeras arose from his successful production by experimental methods of white-margined varieties of *Pelargonium* (vars. *albomarginatae*) (1909). If a pure green variety of *Pelargonium* is fertilised with pollen from a flower growing on a completely white-leaved branch of a variegated plant, the resulting seedlings will show a variegation due to patches of the leaf tissue not developing chlorophyll in their plastids. This is one of the cases of what is known as "mosaic" inheritance. The reason for the absence of chlorophyll is still obscure, and it evidently does not always affect the cell as a whole, for Gregory (1915) had found, in certain variegated Primulas, both yellow and green plastids in the same cell in very young and actively growing leaves (see his Fig. 10, Plate X). But usually the cells will contain either only large green or only smaller yellow plastids. In the case of this leaf-colour therefore, the Mendelian rules of inheritance do not hold good, and we do not obtain a dominant character in the F_1 generation, but both of the apparently allelomorphic characters are inherited, and are distributed irregularly among the cells of the offspring. This character affects not only the leaves, but also the stem. Consequently, if the stem apex falls into a green portion of the tissues, the seedling will grow into a green plant, though its cotyledons will be variegated, while if the stem apex is in a white portion of tissue, the plant will develop into an albino, which will, however, not be viable, as it can only derive an insufficient amount of carbo-

hydrate material from the green patches of its cotyledons. Good figures of these features are to be found in Baur's original paper and are also reproduced in his *Einführung in die experimentelle Abstammungslehre*. From these figures it will also be seen that, if the stem apex arises from a patch of tissue which is partly white and partly green, a sectorially coloured plant will be produced which Baur, using the corresponding term of chimaera suggested by Winkler for such growths, calls a sectorial chimaera. On the other hand, the irregular mosaic patches of white and green tissues do not necessarily occupy the whole depth of the tissue, but one of them may be superficial and a shoot may be developed in which either the potentially green tissues cover in the colourless or *vice versa*. These growths would constitute periclinal chimaeras. If the covering of one of the types of tissue is limited to the epidermis, which is normally devoid of chlorophyll in all Flowering Plants, the periclinal chimaera could not be detected without a microscopic examination to see whether the guard cells of the stomata were green or not. The two plants would look green or white according to the nature of their core. If, however, the skin or covering consisted of two layers of cells of the colourless type then the leaf would appear green in the centre, owing to the fact that the green inner tissue would be visible through the two transparent covering layers of cells. A microscopic examination would show that the uppermost layer of palisade cells contains no green but only colourless plastids. These cells are also generally square and not palisade-like (see Fig. 5). Towards the edge of the leaf where this tissue becomes thinner, the entire thickness may only contain four layers of cells. These would all belong to the colourless constituent of the plant and the leaf would, therefore, have a white margin. Baur's experiments have shown us that such white-margined varieties of plants, which arise spontaneously as side shoots from variegated plants, can be expected to occur if a sufficiently large number of seedlings are raised. We must assume, as Baur indicates, that for the formation of such periclinally variegated shoots the distribution of green and white patches is not necessarily sectorial, but that patches of green tissue and white tissue may alternate on the same radius of the stem. An interesting feature connected with these white-margined varieties is the frequent reversion to a pure green leaf either wholly or in part. This may be due to some destruction in part of the leaf or over the apex of the shoot of the two outermost rows of cells, which are then replaced by regeneration from the "green" core. It may also happen that by proliferation of the outermost two layers a purely white leaf or white shoot is produced. This reversion to the two parental types is, of course, exactly parallel to the segregations met with in the graft hybrids of *Cytisus Adami* and *Crataegomespilus*. It is not to be wondered that Baur was struck by this remarkable correspondence.

A further agreement between the white-margined varieties and the graft hybrids is the result of their sexual reproduction. If they are selfed then, since the reproductive cells develop from the sub-epidermal layer of cells, the offspring are pure greens or whites according to the nature of their sub-epidermal layer. This is exactly paralleled by *Cytisus Adami* producing pure laburnum seedlings, and *Crataegomespilus Asnieresii* producing pure hawthorn seedlings.

The results obtained by Baur as a result of his experiments with *Pelargonium* seemed at first decisive in proving them to be periclinal chimaeras. They were, however, not allowed to pass without serious criticism. Noack (1922), after an examination of the development of the leaf of *Pelargonium*, came to the conclusion that the whole of the mesophyll of the leaf of this plant arises from the sub-epidermal layer of the stem apex. Both the colourless and the green layers of the leaf would have the same origin and could not, therefore, be regarded as derived from parentally distinct tissues. To this criticism Baur replied vigorously as indicated in the section dealing with *Solanum*. The results obtained by Noack were not confirmed but rather contradicted by Schwarz (1927) who, besides dealing with *Solanum*, also examined *Pelargonium*. So did Krumbholz (1925), with the result that he found that different varieties of *Pelargonium* behaved differently as regards leaf development. In the white-margined "Mme Salleray" the leaf was evidently formed by a substantial amount of tissue from the third layer of meristematic cells. In the green form "Distinction," the sub-epidermal layer divides up at a very early stage and it is problematical whether it could form a diplochlamydeous chimaera. Bateson (1924) stated that three of the *Pelargoniums* he examined were not periclinal chimaeras. Krumbholz came to the conclusion that Noack's results might be due to the fact that he examined "green" varieties and also seedlings or young plants in which the conditions in the stem apex may be somewhat different from those obtaining in full-grown plants. Noack then (1924) undertook some breeding experiments with variegated types of *Pelargonium*. Some of his results were in agreement with those obtained by Baur; others, however, showed some differences, but it is mainly on the development of the leaf that he bases his opposition to Baur's interpretation of the white-margined varieties of *Pelargonium*. Noack is thrown back on another explanation of the formation of the white-margined form. He assumes that the cells in their meristematic condition contain a gene determining the colouring matter which is in an "indifferent labile condition," and that this labile condition becomes fixed in one direction or another during the development from the meristematic to the semi-meristematic condition of the cells. In the sexual cells he assumes that the process of determination is already so far progressed that no change in the nature of the gene occurs. Some support is gained for Noack's view from the fact that, in some forms of variegation the leaves undergo a change in the intensity of green colour or grow out of the chlorotic condition, which indicates that it may be of the nature of a diseased condition of the chloroplasts. There are also differences in the colour of the plastids, which may be colourless, yellow, yellowish green or vivid green, giving a wide range of tints to the leaf or parts of the leaf from white through "chlorina" and "aurea" varieties to pure green, which looks as if the protoplasm of the cells controlled the power of the plastids to develop the various colouring matters (carotin, xanthophyll and chlorophyll) which make up the green colour of the leaf. The genetics of some of these variable forms were discussed by Correns (1928) in his address to the International Congress of Heredity in Berlin in 1927. Baur, of course, had considered that as variegation, i.e. the presence and absence of green colour in certain groups of cells, did not follow the ordinary

Mendelian laws the cause of these colour variations did not lie in the genes of the nucleus, but that the appearance of chlorotic cells depended on the presence in the egg cell or the male reproductive cell of chlorotic plastids. This supposition gained confirmation from the fact that Ruhland and Wetzel (1924) were able, by special methods of staining, to demonstrate the existence of plastids in the male generative cells of the Lupin and some other plants. And though it has not been proved for *Pelargonium*, the probability of its being a more general phenomenon is considerable. There is, of course, still some difficulty concerning the subsequent segregation of plastids of one kind only into separate cells, but it must be remembered that Gregory (1915) had already shown that both kinds of plastids may occur side by side in one cell in the case of young leaves of variegated Primulas, and this feature has received confirmation in the case of other variegated plants (Massey, 1928).

While the structure and behaviour of periclinal chimaeras in *Pelargonium* have been studied by many observers such as Bateson (1919), Dahlgren (1921), Chittenden (1925), Noack (1924), Roth (1927) and others, several instances of other variegated plants with a periclinal arrangement of green and white or yellow tissues have been described since Baur's publication on *Pelargonium*. Bateson (1919) has drawn attention to the similar constitution of the white over green and the green over white varieties of *Euonymus japonicus latifolius* var. *variegata*, which correspond very closely in their constitution to the *Pelargoniums* with white or green margins. Chodat (1919) has examined white- and green-margined varieties of *Funkia*, and has found in this plant that their constitution is not quite the same as in the periclinally variegated *Pelargoniums*. In *Funkia*, apparently, the chimaera is haplochlamydeous, the epidermal cells only being of different constitution from the rest of the leaf. As in the case of the epidermis the cells are normally devoid of chlorophyll and the fact of its belonging to a colourless constituent of a periclinal chimaera can only be recognised from the guard cells of the stomata. Chodat finds that in the white-margined varieties of *Funkia Sieboldiana*, the stomata of the epidermal layer, whether covering the white or the green portions of the leaf, are devoid of chlorophyll in their guard cells. These contain colourless plastids which can and do form starch grains, and by their production or transformation into sugar can, therefore, vary the osmotic pressure of the guard cells, which is necessary for the opening and closing of the same. Collins (1922) has shown that variegated forms of *Chlorophytum elatum* and *C. comosum* have a similar constitution. Here, as in *Funkia*, the variegation is generally striated, the margin of the narrow leaves being different in colour from the centre, and as in Monocotyledons, according to Flot (1894), all the tissues of the leaf with the exception of the epidermis arise from a single initial cell or group of cells, only monochlamydeous periclinal chimaeras are to be expected. Indeed, according to Rösler (1928), in grasses the leaves are formed entirely from the dermatogen, so they could not form any periclinal chimaeras at all.

Schwarz's (1927) investigations of the development of the leaves of *Plectranthus fruticosus* and *Ligustrum vulgare* show that, for similar reasons, namely, because the

sub-epidermal layer of the meristem forms the whole of the leaf tissue with the exception of the epidermis, no diplochlamydeous periclinal chimaera can be formed in these plants. Massey (1928) has shown the same to be the case for *Veronica gentianoides*, the form *variegata* with white margins being sectorial in the arrangement of its white and green portions. Thus some Dicotyledons as well as most Monocotyledons are incapable, from the nature of their leaf development, of forming diplochlamydeous chimaeras.

Periclinal as well as sectorial chimaeras have been described for *Acer negundo* by Lakon (1921) and for *Oenothera* by Stomps (1920). Renner, too (1922), obtained variegated offspring in *Oenothera* by crossing green forms of *Oenothera*. This fact had already been noticed by de Vries (1905). From the variegated seedlings produced by crossing Renner obtained both sectorial and periclinal chimaeras. As Ruhland and Wetzel had not shown at the time of Renner's paper the transmission of chloroplasts from the male parent to the egg cell by way of the pollen tube, Renner adopted the view that the protoplasm which passed over with the male generative nucleus exerted some effect on the offspring in addition to the genes of the chromosomes.

Chittenden (1925) has described an interesting white over green chimaera of *Hydrangea hortensis*, in which lobes of solid green, without a white skin, occur at the margin of the leaf. This indicates that certain complications may arise in addition to the primary periclinal arrangement of tissues. In this connection the recent investigations on infectious chlorosis made by Hertsch (1927) show that, in the case of *Euonymus japonica folii aureo-marginatae*, in which the periclinal nature of variegation has been established by Bateson (1919), infectious chlorosis may also occur which naturally modifies the appearance of the plant, variegation from two causes occurring in such specimens.

Bateson (1919), in his *Studies in Variegation*, draws attention to another interesting feature of the periclinally variegated plants. It is not infrequent in some forms to find a leaf or a portion of a leaf in which the tissues show a reversal of the normal arrangement, so that instead of the leaf being white margined owing to the tissues being white over green they become green over white, and thus present a green margin. The terms albomarginatae and albonucleatae have been given to these two forms. What is the cause of this change it is difficult to say. It may be due to some change in the early development of the leaf, and a section figured by Massey (1928) lends support to this view. On the other hand, it might also be explained, according to Noack's suggestion, by the fact that the formation of green and white tissues is not predetermined in the stem apex, but is due to warring tendencies, in which case any of the layers might become white or green respectively. It is not so easy to account for on the periclinal chimaera hypothesis of Baur, nor have we any parallel in the case of graft hybrids.

VIII. CONCLUSION.

The periclinal chimaera theory of the constitution of graft hybrids and of certain forms of variegation may be extended to other characters in plants in which no

such constitution has been anticipated. Bateson (1916, 1921) has shown that plants which lend themselves to propagation from root cuttings, in which case they produce adventitious buds from internal tissues, do not always come true to type. Cramer (1907) had already drawn attention to this fact, and Bateson, experimenting with *Bouvardias*, found a double pink variety "Bridesmaid" invariably produced from root cuttings a red double "Hogarth." In other *Bouvardias* similar changes took place, but not invariably so. Though at first not successful with *Pelargonium* Bateson later, using methods elaborated by that very successful propagator Mr Stewart of the Edinburgh Botanic Gardens, was able to obtain, from the roots of several fancy *Pelargoniums* such as "Pearl," "Mrs Gordon" and "Escot" by budding, plants which differed from the parental forms primarily in flower colour. In some cases they differed also in the development of their reproductive organs, and in other respects. The leaves also were flat, not crumpled as those of the parental form often are. Chittenden (1927) has found that the root cuttings of "Double New Life," which is devoid of anthers, produce a normal single flower hermaphrodite. As Bateson points out in his Joseph Leidy Memorial Lecture (1926), in most of the examples the inner component may be regarded with some plausibility as differing from the outer in the possession of a dominant character; but in one example, a double magenta *Pelargonium*, the two root cuttings raised have both been reds. Red may, therefore, be taken to be the inner component and, judging from analogy, is probably the recessive. But though the constant appearance of a particular type from root cuttings must indicate that the arrangement of the somatic layers is orderly and probably periclinal when different forms arise, sometimes like the parental form, sometimes differing from it as in the *Bouvardia* "Vulkan," we may have to assume a mosaic arrangement of tissues. Indeed in the salmon-fringed *Pelargonium*, which is undoubtedly of periclinal structure, flowers are often a patchwork of the outer and inner components, as happens sometimes with periclinally variegated leaves.

Åkerman (1920, 1927) has described chimaeric features in connection with several cultivated forms of wheat. These speltoid chimaeras, as he calls them, are probably only cases of somatic segregation of heterozygous forms of common wheat. They vary in the amount of tissues which they affect, and may be limited to epidermal cells. It is questionable whether such segregation phenomena should be called chimaeras. The same applies to the sectorial chimaera of an apple described by Lamprecht (1927). In this case a sector with the colouring of a Ribston Pippin was found on a Cox's Pomona apple. Since the latter variety is said to have been derived from the former type we have probably a bud variation reverting to an ancestral type. The same may probably be said of the chimaeric apple described by Dahlgren (1927). On the other hand, the apple described by Castle (1914) is of a different nature. It is said to have resulted from the grafting of Golden Russet on Boston Stripe, and the fruits are said to have resembled the former variety at their base and the latter at their upper end. Further investigation of this case is very desirable.

Other phenomena, such as the occurrence of ever-sporting varieties of flowers, may in some cases at all events be explained as chimaeras. Indeed Chittenden (1928)

indicates that the behaviour of certain ever-sporting races of *Myosotis* are strongly suggestive of a chimaeric structure. In this case, possibly, we may have also to deal, as in the case of variegated leaves, with dissimilar plastids, for Chittenden has shown (1927) that plastids may be directly concerned with anthocyanin production. Other ever-sporting varieties of flowers may, on further experimental investigation, turn out to be of a chimaeric nature.

The renewed interest which was awakened in the study of graft hybrids early in the present century, no doubt in association with the revival in that of genetics brought about by the "rediscovery" of Mendel's work, has led to a considerable development of our views as to their nature. Cytology, morphology and genetics have contributed towards the progress. Winkler's experimental work in the production of graft hybrids in *Solanum* and Baur's experimental work with *Pelargonium* have been particularly helpful, and the suggestion that both graft hybrids and white-margined leaves should be regarded as periclinal chimaeras, while it has produced plentiful criticism, has also suggested new lines of investigation, and has proved to be as fruitful as any fundamentally new conception usually is, whether it ultimately stands the test of time or not.

There is no doubt that the problem of graft hybrids is by no means completely solved, though we have a better conception now of the possibilities of these curious formations. Some, no doubt, probably most of those we know, are periclinal chimaeras, and it may yet be shown that all of them are of this nature. Still there are obvious difficulties in bringing *Solanum Darwinianum*, the *Crataegomespili* and *Pirocydonia* into complete agreement with the chimaeric theory, though certain characters point in that direction. If they are to be included in this category we must, at all events, allow for a far greater interaction and consequent modification of the component tissues than was at first thought to be the case. On the other hand, there is no *a priori* impossibility of the fusion of vegetative cells in grafting. Winkler has shown experimentally that the nuclei of vegetative cells of the same plant can fuse to form a tetraploid individual. No doubt it would be more difficult for the vegetative cells of plants belonging to different species to unite in this way, particularly if, as in the case of *Cytisus purpurens* and *Laburnum vulgare*, the generative nuclei cannot be induced to unite. In the case of *Crataegus* and *Mespilus*, on the other hand, which are known to produce seed hybrids, it may be that the vegetative nuclei have united and thus have produced a true graft hybrid. Obviously this can at present be only hypothetical, and the fact that certain presumptive graft hybrids have turned out to be periclinal chimaeras lessens the probability. Still the question remains an open one. The only clearly demonstrated case of the fusion of protoplasm of vegetative cells is to be found in the experiments of Burgeff (1912), who obtained the union of protoplasm of mycelia of + and - strains of the fungus *Phycomyces*, and thus produced a neutral plant. In this case the two vegetative strains had already a tendency to unite. Perhaps grafting experiments with higher plants of dioecious habit might be successful in producing a true graft hybrid, as one might presume a greater tendency of fusion of the vegetative tissues of a male and a female plant.

There are other promising lines of research open in this field of investigation

as Winkler points out in his *Chimaerenforschung*, 1913. Among these one of the most interesting and one which has already attracted the attention of several workers, is the question of the possible immunity from attack of parasitic organisms, when the core of a plant is protected by the epidermis of another species or variety. Daniel had already pointed out (as mentioned above) that his *Pirocydonia Danieli*, though attacked by *Gymnosporangium Sabinae* and showing reddish spots, was sufficiently resistant to prevent the formation of aecidia, owing this resistance to the quince constituent which is immune to this fungus. *Pirocydonia Winkleri*, which he regarded as a "hybride de greffe renforcé," having accentuated characters of the quince, is immune to this fungus.

Klebahm (1918), experimenting with *Septoria lycopersici* on Winkler's *Solanum* chimaeras, found *Solanum proteus* to be severely attacked, its leaves being, as we have seen, largely composed of tissues of the tomato. *Solanum tubingense*, as one might expect, was only slightly attacked, as its mesophyll is mainly composed of nightshade. *S. Koelreuterianum* was severely, but *Gaertnerianum* only slightly, attacked. The results, therefore, are quite in accord with the periclinal structure of these chimaeras. Fischer (1912) inoculating the *Crataegomespili* with *Gymnosporangium clavariaeforme*, which attacks the hawthorn, but to which the medlar is immune, obtained equally striking results, *Crataegomespilus Asnieresii* being slightly affected by this fungus, by the covering epidermis of the medlar, while *Crataegomespilus Dardari* in which the leaf is presumably largely composed of *Mespilus* tissues was quite immune. Anna Maurizio (1927), in a critical account of the susceptibility of the hybrids of the Pomaceae to parasitic fungi, has obtained interesting results, but is a little more guarded in her conclusions, interpreting her results as compatible with a burdo-like structure of the graft hybrids. Jörgensen's experiments to obtain a periclinal chimaera with a core of the potato surrounded by a skin of the tomato, in which we have seen he was unsuccessful owing to the reluctance of the potato to form adventitious buds, were undertaken with a view to ascertain whether the potato, so important an economic plant, could be protected from the attacks of late blight (*Phytophthora infestans*) by a protective skin of tomato epidermis. Possibly this result may still be achieved. If so this protective epidermis might also be defensive against the attacks of the green fly, and thus mitigate the enormous losses to our potato crops by the various virus diseases of that plant which are now receiving intensive investigation. Since potatoes are commercially propagated by tubers, if a foreign skin does not interfere with tuber formation, a very real protection of the industry might be effected in this way.

This review of the present position regarding graft hybrids and chimaeras deals only with the phenomena as they present themselves in the vegetable kingdom. Less has been done in the animal kingdom in this direction, as grafting is not a universal practice, as it has been from time immemorial in horticulture. Of recent years, however, the transplantation of animal tissues has become a subject of experimentation, particularly among the lower forms of life. In this way, by grafting of portions of the tissues of different species or varieties, chimaeric organisms sometimes of

Echinoderm larvae by Bierens de Haan. The term "animal chimaeras" was suggested by Spemann in 1921 when he succeeded in uniting tissues in early developmental stages of *Triton taeniatus* and *Triton crisbatus*. Graftings of other Amphibia have also been obtained, and *Hydra* has been frequently used for grafting experiments as, for example, by Goetsch (1923, 1924), by Issayew (1923, 1924) and by Mutz (1930). These authors should be consulted for further references to animal chimaeras.

IX. SUMMARY.

In the introductory paragraphs of this review, the question was discussed whether after grafting, any effect of scion on stock or *vice versa* was known to occur. It was indicated that apart from the infectious chlorosis, a supposed virus disease, any influence of stock on scion or scion on stock is generally regarded as non-existent. Professor Daniel, however, who has had unrivalled experience of grafting, holds the opposite view and believes strongly in the intermingling of characters of stock and scion (hybridation asexuelle) caused particularly by his method of mixed grafting.

The earliest graft hybrid which has been scientifically investigated, the so-called "Bizzarria" Orange, is then described. It is regarded by some as a true graft hybrid, by others as a synthetic chimaera of periclinal constitution. Other instances of sectorially diverse oranges may be due to partial segregation of heterozygous forms of Citrus.

The well-known and much discussed *Cytisus Adami* seems according to the investigations of Buder (1910 and 1911) to be what Baur (1910) has called a periclinal chimaera with a core of the Common Laburnum surrounded by an epidermal layer of *Cytisus purpureus*.

Though according to Baur (1919) and Meyer (1915) the *Crataegomespili* are also periclinal chimaeras with a core of the hawthorn surrounded by one or more layers of the medlar, the more recent investigations of Haberlandt (1926) and Weiss (1925) as well as previous observations by Daniel (1914) throw some doubt on the chimaeric structure of these so-called graft hybrids.

The constitution of *Piroydonia* is still uncertain and the *Amygdalopersica* described by Daniel (1914) seems likely to be a heterozygous plant.

Of the various forms of growth obtained by Winkler by grafting tomato on nightshade stock and *vice versa*, four seem to be definitely proved to be periclinal chimaeras, but the constitution of *Solanum Darwinianum* is still doubtful, and it may possibly be a true graft hybrid formed by a union of vegetative cells.

The formation of chimaeric growths of variegated plants both of sectorial and periclinal constitution was experimentally established by Baur (1909) for *Pelargonium* and further investigated by Bateson (1919 and 1920), Chittenden (1925) and others. This has led to interesting discussions and observations concerning the part played by the plastids in the reproductive processes of plants and generally in the inheritance of variegated leaves.

A further point of interest is the suggestion made by Chittenden (1930) that certain eversporting forms of flowers may be of chimaerical constitution.

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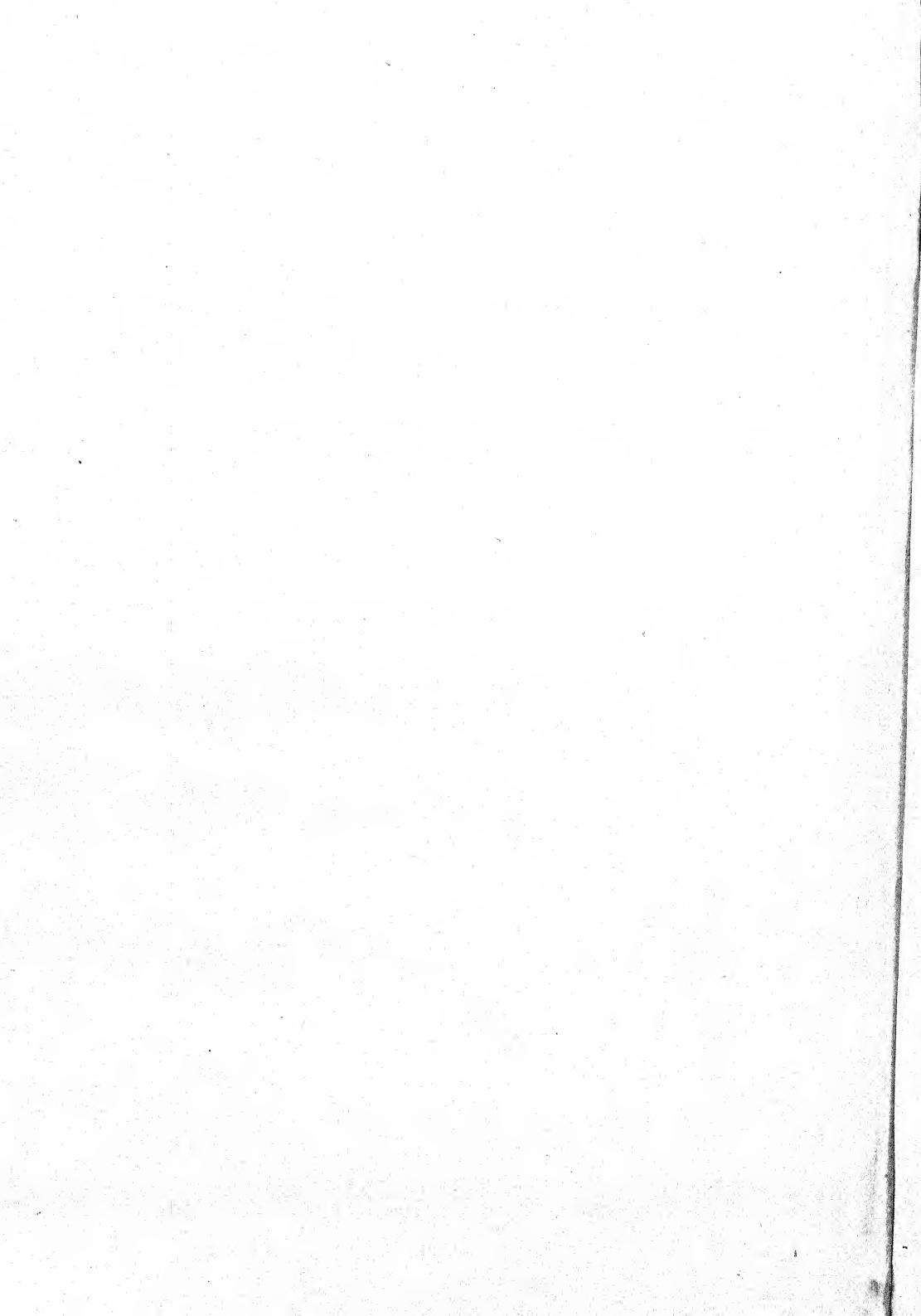
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THE RÔLE OF CELLULOSE IN NUTRITION

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(Received March 31, 1930.)

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I. INTRODUCTION.

A FATTENING bullock of 11 cwt. live-weight, subsisting on a ration composed of meadow hay, oat straw, silage, dried sugar beet pulp and concentrates, may consume as much as 6 lb. of fibre daily. The question of the part which fibre plays in the nutrition of animals is, therefore, one of extreme importance to the feeder of live-stock. Is the function of the bulky, fibrous food merely that of filling the capacious rumen of cattle and sheep, conferring thereby the desired feeling of contentment and repletion after feeding? Or, on the other hand, is the fibrous constituent of feeding stuffs able to undergo, during digestion, transformation into nutrients capable of contributing to maintenance and production in the animal?

It is the purpose of the writer to summarise the evidence on which the agricultural chemist has based his answers to these and kindred questions. If, then, the evidence to be examined in this review is mainly that derived from the work of the agricultural investigator, it is hoped, nevertheless, that the account may prove of help and interest to workers in other domains of biology, who are engaged, no less usefully, in the study of cellulose from many widely differing standpoints.

II. CHEMICAL NATURE OF CELLULOSE AND ALLIED COMPOUNDS.

Cellulose is a member of the class of carbohydrates known as polysaccharides. It appears to be produced by the polymerisation of a simple glucose anhydride unit; its molecule, therefore, is represented by the formula $(C_6H_{10}O_5)_n$. By complete hydrolysis with acids it gives rise to glucose, and in this respect resembles starch. Whereas starch, however, is readily broken down by the action of dilute acids, the hydrolysis of the more stable cellulose complex requires the use of strong acids.

That cellulose is not a polymer of starch, nor, indeed, structurally related to it, is brought out by a study of the products of its enzymatic hydrolysis. Diastatic hydrolysis of starch gives rise to the disaccharide, maltose (glucose- α -glucoside), which, by the further action of maltase, passes over into glucose. Hydrolysis of cellulose by cytases, on the other hand, leads to its transformation into the disaccharide, cellobiose (glucose- β -glucoside). Maltase is without action on cellobiose, the latter undergoing hydrolysis to glucose by the action of cellobiase. It may be concluded, therefore, that cellulose and starch are fundamentally distinct substances and are elaborated in the plant by two different mechanisms.

The fibre in plants and feeding stuffs rarely consists solely of cellulose. The latter is usually admixed with varying amounts of compounds of a more resistant and carbonaceous type, known as lignocelluloses. Small amounts of the more stable and resistant pentosans may also be present. These so-called incrusting substances are formed in the cell walls of plants during the process of lignification, and they serve to confer a degree of rigidity on the growing plant. The higher the proportion of lignocellulose in the fibre, the more closely does the fibre approach wood in nature.

The estimation of the fibre in feeding stuffs and plant products is carried out by boiling 2 to 3 gm. of the material with 200 c.c. of 1½ per cent. sulphuric acid for 30 minutes, filtering off the acidic reagent through linen and boiling the residue for a similar period with 200 c.c. of 1½ per cent. potassium hydroxide solution. This treatment removes the protein, oil, soluble carbohydrate and soluble ash, the residue consisting, therefore, of fibre and silica. The loss in weight on igniting the dried residue represents the amount of fibre in the weight of material taken for the estimation.

III. THE DIGESTIBILITY OF CELLULOSE.

It was at one time thought that any food constituent which resisted being brought into solution by boiling successively with dilute acid and alkali would also escape digestion in the animal. This is now known to be by no means the case. Farm animals, more particularly ruminants like sheep and cattle, are able to digest and assimilate large portions of the fibre contained in their rations.

The extent to which animals are able to digest the fibre of their food is determined by digestion trials, usually carried out on wether sheep and, less frequently, on bullocks and pigs^(1, 2). The animal is kept in a metabolism crate during the trial and is fitted with a light harness which enables urine and faeces to be collected separately. During the experiment, lasting from a fortnight to three weeks, the

animal receives a weighed ration of known fibre content. The faeces are collected, weighed and analysed, the fibre content being determined by the same method as is employed for the fibre in the feeding stuff. By subtracting the mean daily output of fibre in the faeces from the amount of fibre in the daily ration, the amount digested and assimilated is ascertained. From these data is calculated the percentage digestibility, or digestion coefficient, of the fibre in the food.

That cellulose, contrary to early opinion, may be digested and assimilated by animals was first shown by Haubner in 1855, in experiments on cattle (3). His results were quickly confirmed by Henneberg and Stohmann (4), while a long series of subsequent digestion trials has established beyond all question the fact that cellulose is, to a greater or less extent, digested both by herbivora and omnivora.

(a) *Ruminants (sheep, cattle and dairy cows).*

Ruminant animals have the greatest power of digesting and utilising the fibre in feeding stuffs. They do not, however, digest the fibre in all feeding stuffs to an equal extent. The fibre in a young green crop, for example, is digested much more completely than the fibre in the same crop after it has grown to the seeding stage. To understand the reason for this, it is necessary to consider the nature of the changes which occur in the plant as it progresses towards maturity.

In the young plant, the cell walls are extremely thin and tender, and are composed, almost entirely, of pure cellulose. The latter is digested in the ruminant tract to almost the same extent as is the carbohydrate fraction. In determinations of the digestibility of pasture herbage in its early stages of growth, for instance, it has been shown that the digestion coefficients of the fibre and the carbohydrate are 84 and 87 per cent. respectively (5, 6, 7, 8). This and other experiments have demonstrated that, for ruminants, pure cellulose is a nutrient of high digestibility.

As the plant matures, the cell walls become thickened and toughened, owing to their becoming surrounded by, and intimately admixed with, the much more carbonaceous and woody form of fibre known as lignocellulose. The latter is entirely indigestible. It follows, therefore, that the process of lignification is accompanied by a gradual diminution of the digestibility of the fibre in the crop. Thus, whereas the fibre in grass in its early stages of growth is 84 per cent. digestible, that contained in grass at the hay stage of maturity is digested to the extent of only 52 per cent.

But the consequences of lignification are more far-reaching than this. Unless the fibrous cell walls are capable of readily being digested away in the rumen of the animal, the food nutrients like protein, oil and carbohydrate, contained within the cells, may not become easily accessible to the action of the digestive ferments in the alimentary tract. For this reason, the protein and carbohydrate in lignified fodders like hay and straw are only poorly utilised; whereas in non-lignified fodders, like young, leafy pasturage, where the cell walls can easily be digested away, and where the food nutrients are readily liberated from the cells to the action of the digestive enzymes, the protein and carbohydrate are digested and assimilated to an unusually high degree. The consequences of lignification, in so far as they influence the digestibility of the fibrous and non-fibrous constituents of a crop, are brought out clearly by the results

recorded in Table I. These results were obtained in the pasture investigations to which reference has already been made (5, 6, 7, 8).

Table I. *Digestion coefficients of constituents of grass in its young, leafy stage of growth and at the hay stage of maturity.*

	Young, leafy grass (%)	Grass at hay stage of maturity (%)
Fibre	84·2	52·4
Carbohydrate	87·4	53·0
Protein	85·4	50·0
Oil	60·0	30·0

The digestibility of fibre, therefore, is inversely proportional to its content of lignocellulose. This statement is further borne out by the data summarised in Table II, showing the results which have been obtained in determinations, with sheep, of the digestibility of the fibre in certain lignified and non-lignified feeding stuffs.

Table II. *Digestion coefficients of cellulose in non-lignified and lignified feeding stuffs (trials with sheep).*

	Digestion coefficient of cellulose (%)
Non-lignified feeding stuffs:	
Wet sugar beet pulp (9)	89·8
Dried sugar beet pulp (9)	89·7
Mangolds (10)	78·0
Cabbage (11)	74·0
Sugar beet tops (12)	71·0
Lignified feeding stuffs:	
Oat straw (11)	54·0
Wheat straw (11)	50·0
Undecort. cotton cake (11)	37·0
Linseed cake (11)	32·0
Wheat bran (11)	26·0
Palm kernel cake (13)	21·0

Kellner (14) showed that if the incrusta in straw (silica and lignocellulose) were removed by treatment with alkaline reagents, then the digestibility of the cellulose in the residual product was extremely high. 1000 kg. of rye straw was boiled for 3½ hours, under 7 atmospheres pressure, with 2070 litres of a solution containing, per litre, 55 gm. sodium hydroxide, 20 gm. sodium carbonate and 22 gm. of sodium sulphite and sodium thiosulphate. At the end of this period the residual material was filtered off and well washed with water. The fibre in this so-called "Strohstoff" was digested by cattle to the extent of 95·8 per cent. Kellner attributed this high digestion coefficient to the removal of the incrusting substances and to the circumstance that the "Strohstoff" was fed in a very finely divided condition. This discovery was made use of in Germany during the Great War for enhancing the feeding

value of straw by treatment with solutions containing sodium hydroxide, sodium carbonate or quicklime (15).

It has also been demonstrated that storage of a green crop in the tower silo leads to a definite increase in the digestibility of the fibre in the crop. In an investigation into the comparative feeding values of green oats and vetches and oat and vetch silage, Woodman (16) showed that ensilage had increased the digestibility of the fibrous constituent of the crop from 47·6 to 57·1 per cent. In a similar investigation with the maize crop, an increase from 65 to 70 per cent. in the digestibility of the fibre was noted (17). It is probable that the loose association between the cellulose and the incrusting substances is broken down by the action of the organic acids which arise during the ensilage of the crop. Since green crops in general contain large amounts of fibre, the increase in digestibility of this constituent brought about by ensiling such crops is of great economic significance.

(b) *Swine.*

Digestion of cellulose in the ruminant organism is brought about mainly in the capacious paunch or rumen. Since the digestive tract of pigs is of much simpler construction, it is customary to assume that swine are incapable of digesting the fibre of feeding stuffs with the degree of efficiency displayed by ruminants. This is true with feeding stuffs in which the fibre is present in the lignified condition; but with non-lignified feeding stuffs swine are able to digest the fibrous constituent almost as completely as can sheep and cattle. It may be concluded, on the basis of the data recorded in Table III, that pure cellulose, freed from incrusting materials, is readily digested by pigs, but that the latter cannot make such good use of lignified fibre as can ruminant animals.

Table III. *Digestion coefficients of cellulose in non-lignified and lignified feeding stuffs (trials with pigs).*

	Digestion coefficient of fibre	
	Pig trials (%)	Sheep trials (%)
Non-lignified feeding stuffs:		
Sugar beet (18)	90·1	—
Molasses-sugar beet pulp (19)	84·4	—
Dried sugar beet pulp (19)	84·3	89·7
Dried potato slices (11)	80·0	—
Mangolds (11)	79·0	78·0
Lignified feeding stuffs:		
Maize meal (11, 20)	23·0	58·0
Barley meal (21, 2)	10·8	55·0
Dried brewer's grains (11)	15·0	48·0
Linseed cake meal (11)	12·0	32·0

(c) *Horse.*

Horses are also able to digest the fibre of feeding stuffs, the digestion occurring mainly in the enormous caecum. In general, however, comparisons show that the

capacity of the horse to deal with fibre is not so great as is the case with ruminants, a statement which is illustrated by the figures given in Table IV (22).

Table IV. *Digestion coefficients of cellulose in feeding stuffs (comparison of results with sheep and horses).*

Feeding stuff	Digestion coefficient of fibre	
	Trials with sheep (%)	Trials with horses (%)
Meadow hay	56·0	36·0
Oats	30·0	21·0
Beans	79·0	65·0

(d) *Birds.*

Birds are regarded as having no power of digesting cellulose, even though fibre forms a part of their normal diet. Although poultry are distinctly inferior to other farm animals in their ability to assimilate cellulose, the power is not, however, entirely absent. In recent feeding trials with White Leghorn cockerels, the digestion coefficient of the fibre in Sussex ground oats was found to vary from 2·5 to 11·9 per cent., with a mean value of 7·6 per cent. (23)

(e) *Man.*

The less resistant forms of cellulose found in the human dietary appear to be digested fairly readily in the human tract. It has been demonstrated in trials on human subjects that from 30 to 76 per cent. of the cellulose in the food may be digested and assimilated (24).

(f) *Invertebrates.*

The power of certain invertebrates to utilise cellulose is very high, as is made clear in the following summary taken from a recent *Report of the Medical Research Council* (25): "Among the invertebrates, certain gastropods, notably *Helix pomatia*, the land snail, digest cellulose. Many insect larvae can digest large quantities of woody material and cellulose, and this faculty is retained in some of the imagines. White ants can live almost solely upon cellulose, and with such effect, that in their millions they can demolish a house or a forest tree in a space of hours, and have made civilised life an impossibility in some parts of the world. The woodworm in the antique mahogany dining-table is perhaps a more homely example of successful cellulose digestion among lower animals."

IV. THE FOOD VALUE OF CELLULOSE.

Our knowledge of the value of cellulose in the nutrition of farm animals is derived from the results of Kellner's classical investigations published in 1900 (26). The problem which confronted Kellner was to devise a method for measuring the energy available in feeding stuffs for productive purposes in the animal. For this

purpose he used an apparatus known as a respiration chamber. This is essentially an air-tight chamber, provided with inlet and outlet pipes, and large enough to accommodate a bullock in comfort. Air is drawn at a measured rate through the chamber, and from time to time its content of water vapour, carbon dioxide and methane is determined. The animal is provided with a harness which permits of separate collection of urine and faeces. By such means, it is possible to measure the daily output of liquid, solid and gaseous excreta under the experimental conditions.

Kellner's procedure in his experiments with fattening cattle was to determine, in the first place, how much food, such as meadow hay, clover hay or oat straw, was required to maintain the weight of the bullock unchanged. In none of his experiments, however, was it possible to feed the bullocks so that their weights remained absolutely constant over the whole period of experiment. Some animals gained a little fat or flesh, while others lost a little; but as the amounts gained or lost could be determined accurately by this balance method of experimentation, it was possible to make the necessary allowances in assessing the true requirements for maintenance, and, further, to introduce correction factors in the subsequent feeding periods when the fat-producing capacities of the concentrated feeding stuffs were investigated.

Having determined the size of the bullock's maintenance ration, Kellner then carried out a second period of feeding in which a definite weight of concentrated food was added to the maintenance ration. The average daily output of the various forms of excreta was determined, and the mean daily carbon and nitrogen balances were ascertained. With the help of these data, Kellner calculated how much fat was formed in the body of the animal when a given weight of concentrated food was added to the maintenance ration. For convenience, the small amount of protein stored as flesh was converted into its isodynamic weight of fat by multiplying by the factor 5·7/9·5 (*i.e.* the ratio of the heats of combustion of body protein and fat) and was added to the weight of pure fat to give the total amount of production in the animal.

The case of linseed cake may be cited as an illustration of this type of balance experiment. Kellner found that if linseed cake were fed as a supplement to the maintenance ration, then fat (*i.e.* pure fat plus protein expressed as fat) was formed in the body of the bullock at the rate of 18·84 lb. per 100 lb. of cake. In a further experiment he demonstrated that the replacement of the oil cake by starch led to fat production at the rate of 25 lb. per 100 lb. of digestible starch. It followed, therefore, that 100 lb. of linseed cake had a fattening value, when fed in conjunction with maintenance rations, equal to that of 75·36 lb. of starch. The latter figure, therefore, was the starch equivalent of linseed cake.

By repetition of such experiments, Kellner was able to evaluate the starch equivalents of a large number of feeding stuffs, and in this way to obtain a simple numerical comparison of their productive values. Naturally, however, the ability to determine starch equivalents was dependent on the possession of a respiration chamber, a costly and an elaborate instrument of which only a limited number has been built. In order, therefore, to render his method more widely applicable,

Kellner worked out a second method whereby the starch equivalent of a feeding stuff could be calculated from a knowledge of its content of digestible nutrients. He determined how much fat was produced in the body of a bullock when the common food nutrients such as starch, sugar, protein (in the form of gluten of known digestibility), fibre (in the form of the finely divided "Strohstoff" prepared from rye straw in the manner already described), and oil from various sources, were added to the maintenance ration. The results of these experiments are recorded in Table V.

Table V. *Fat-producing capacities of digestible food nutrients.*

Digestible nutrient	Lb. fat production per lb. of digestible nutrient	Starch equivalent of 1 lb. of digestible nutrient
Starch	0·248	1
Fibre	0·253	1
Cane sugar	0·188	0·76
Glucose	0·188	0·76
Protein	0·235	0·94
Oil from oily seeds and oil cakes	0·598	2·41
Oil from cereal grains and by-products	0·526	2·12
Oil from coarse fodders and roots	0·474	1·91

The result in Table V which is of particular interest in connection with the subject under review is the finding that digestible fibre has a value equal to that of digestible starch for fat production in the ruminant animal. The fibre in the finely divided "Strohstoff" was digested to the extent of 96 per cent., and every lb. digested produced as much fat in the body of the bullock as was produced by an equal weight of digested starch. This finding is given practical expression in Kellner's well-known and well-tested formula for calculating the starch equivalent of a feeding stuff from its digestible composition, an equal value being ascribed to digestible fibre and digestible carbohydrate.

In natural feeding stuffs, however, the fibre is not present in the form of pure, finely divided cellulose. The latter is embedded in incrusting materials, such as silica and lignocellulose. The animal must expend energy in masticating the indigestible portion of the fibre, in separating the cellulose from the incrusta during digestion and in passing the indigestible residues along the alimentary tract for excretion. The expenditure of energy for these purposes reduces, in a greater or less degree, dependent on the digestibility of the feeding stuff, the amount of energy which the animal would otherwise be able to derive from the cellulose and the other digestible nutrients. For this reason, if the starch equivalent of a feeding stuff, of known digestible composition, be calculated on the basis of the starch equivalents of the pure nutrients, and the value be checked against that obtained by actual experiment on a bullock in a respiration chamber, the latter figure is always smaller than the value predicted by calculation. This discrepancy is of widely varying magnitude for different feeding stuffs. In the case of linseed cake, for example, the experimental figure is 97 per cent. of the calculated value; for barley meal, 98 per cent.; for swedes,

85 per cent.; for bran, 77 per cent.; for good meadow hay, 67 per cent. and for oat straw, 43 per cent.

It will be noted that the discrepancy between the calculated and experimental values is, as might be anticipated, roughly proportional to the fibre content of the feeding stuffs. In his investigations into the fat-producing capacities of different hays and straws, Kellner found that every lb. of fibre caused the true starch equivalent to be 0·58 lb. less than that calculated on the basis of digestible composition. When calculating the starch equivalent of a coarse fodder like hay or straw, therefore, it is customary to add together the starch equivalents of the digestible nutrients (assuming an equivalence between digestible fibre and digestible starch) and to subtract from this value 0·58 times the percentage of crude fibre in the fodder.

The following conclusions may be drawn from the results of Kellner's investigations: If 1 lb. of pure, finely divided cellulose be added to the maintenance ration of a bullock, it will form as much fat in the body of the animal as would be produced by feeding 1 lb. of starch or other forms of digestible polysaccharide. The starch equivalent of digestible cellulose is unity. Under ordinary conditions of feeding, however, when the cellulose is embedded in indigestible fibre, the net starch equivalent derived from 1 lb. of digestible fibre is less than unity, since a deduction must be made on account of the energy used up by the animal in masticating the indigestible portion and in separating the cellulose from the incrusta.

V. MECHANISM OF DIGESTION OF CELLULOSE.

Any theory which is put forward to explain the breakdown of cellulose in the ruminant tract must be compatible with the experimentally demonstrated fact that the products of such digestion of a given weight of digestible cellulose are equal in nutritive value to the products derived from the digestion of the same weight of digestible starch.

The digestion of starch is brought about by enzymatic activity, the starch being hydrolysed to maltose, by the ferments ptyalin and amylopsin, and ultimately to glucose by the further action of maltase. In addition to changes produced by enzyme action, however, carbohydrates like starch are also subject to attack by bacteria in the rumen of the animal. As a consequence of such activity, a portion of the carbohydrate in the food is fermented with the production of methane and organic acids, such as acetic and butyric acids. This destructive fermentation explains why the physiological heat value of digestible carbohydrate (namely, 3760 cals. per gm.) is less than its heat of combustion (namely, 4100 cals. per gm.).

The hydrolytic breakdown of the stable cellulose molecule by means of enzymes has not been elucidated with the same thoroughness as has the corresponding process with starch. Such cellulose-hydrolysing enzymes, however, known as cytases, have been shown to be present in seeds and other plant tissues. It has been demonstrated⁽²⁷⁾ that the breaking down of the cell wall of the endosperm cells, whereby nutrient matter is released for the nutrition of the embryo in the earliest stages of growth, is brought about by the action of a cellulose-dissolving enzyme which, though not existent in the resting seed, is produced during the process of

germination. Extracts of this enzyme are able to effect with rapidity the disintegration of the parenchymatous tissue of the potato, carrot, turnip, etc. A cyto-hydrolytic enzyme of great activity has also been shown to be present in the hepato-pancreatic secretions of several species of land snails (28) and in the saliva of certain insects (29).

Up to the present, however, investigation has failed to reveal the production of cyto-hydrolytic enzymes by the digestive glands of the higher animals, and it is safe to assert that the breakdown of cellulose in the ruminant tract is not the work of a cytase present in any of the digestive secretions. At the same time, however, the possibility of the presence of such enzymes in certain types of plant food, for example, cereal grains, should be borne in mind. Under the favourable conditions provided by the alimentary canal, cytases might conceivably become active in such plant material and cause the hydrolytic breakdown of cellulose to cellobiose and glucose. It has been shown, for example, that the starch of maize, fed in the dry condition, is only slightly less digestible in the pig than the starch of cooked maize, and from this finding it has been concluded that the cellulose coatings of the starch cells are easily dissolved away during digestion. It is by no means improbable that the removal of the cellulose pellicles is effected by a cytase in the grain which becomes active under the conditions of the digestive tract. This possibility is strengthened by the fact that the trials were carried out on non-ruminant animals (30).

The presence of a cytase in considerable amounts has been demonstrated recently in the stomachs of pigs and cows, the enzyme in all probability having entered with the food (31). It is also considered probable that the power of reindeer to subsist on lichens and mosses depends on the hydrolysis, during digestion, of the cellulose by cytases pre-existent in the food, since arctic animals have been found to contain few, if any, intestinal bacteria (32).

In the higher animals, however, it is now the accepted belief that the digestion of cellulose is mainly brought about by bacteria.

Tappenheimer (33) seems to have been the first, in 1884, to show experimentally that the disappearance of cellulose in the digestive tract is effected by a fermentation brought about by the micro-organisms inhabiting the alimentary canal. His conclusions have been fully confirmed by more recent investigations, notably those of Markoff (34), while Kellner (35) has shown that the consumption of straw pulp by cattle causes a marked increase in the amount of methane eliminated. The digestion of cellulose occurs chiefly in the parts of the alimentary tract where the food stagnates. In ruminants, the main seat of fermentation is the rumen, while in the horse, with its relatively small, simple stomach, it takes place principally or wholly in the caecum and colon. In the pig, cellulose fermentation occurs in the caecum and colon; in man, the presence of cellulose-splitting bacteria has been detected in all parts of the small intestine (35).

VI. PRODUCTS OF DIGESTION OF CELLULOSE.

When digestion of cellulose is brought about by autolysis, *i.e.*, by the action of cyto-hydrolytic enzymes pre-existent in the food, the products of such digestion are the sugars cellobiose and glucose, resulting from direct hydrolytic cleavage of the

cellulose complex. The products of bacterial fermentation of cellulose, however, are quite different, as is apparent from the studies of the characteristics of cellulose-splitting bacteria which have been made by numerous investigators.

Omelianski (36), working in artificial media seeded with cultures of the bacteria, has demonstrated the existence of two distinct anaerobic cellulose-fermenting organisms. The first breaks down cellulose to *hydrogen* and carbon dioxide, together with organic acids, among which were noted acetic, butyric and valeric acids, with traces of the higher fatty acids. The products of decomposition of the second type of organism are *methane* and carbon dioxide, together with acetic and butyric acids. Kellermann and McBeth (37) claimed that the organisms described by Omelianski were not pure cultures and demonstrated further that cellulose may be decomposed under aerobic conditions. They isolated 36 active species from various sources, all of which rapidly decomposed cellulose and other carbohydrates with the production of organic acids *but no gas*.

More recently, Viljoen, Fred and Peterson (38) isolated a thermophilic organism in pure culture which destroys cellulose at 65° C., forming mainly acetic acid, together with small amounts of butyric acid and ethyl alcohol, and the gaseous products carbon dioxide and hydrogen. The range of temperature over which the organism attacks cellulose is from 43 to 65° C. It lives at 38° C. and 72° C., but does not ferment cellulose at these temperatures. The spores are very resistant to heat and withstand 115° C. for 35 minutes. Heating the spores to 100° C. for 5 to 10 minutes leads to an increased rate of germination.

In addition to cellulose, the micro-organisms also ferment starch, raffinose, sucrose, maltose, lactose, mannose, galactose, fructose, glucose, xylose and arabinose. The presence of organic nitrogen appears to be necessary for the fermentation, and this is best supplied in the form of peptone. The amount of cellulose destroyed in 1 to 5 per cent. suspension varies from 70 to 95 per cent. 50 to 55 per cent. of the cellulose thus broken down is regained as acetic acid, 5 to 25 per cent. as ethyl alcohol, and the rest as small amounts of butyric acid, carbon dioxide and pigment. The pigment is a fatty substance, soluble in ether. The fermentation begins 12 to 18 hours after inoculation and is soon stopped by accumulation of acid. It proceeds continuously, however, if an excess of chalk be added to the medium to preserve neutrality. The micro-organism was derived from an infusion of 100 gm. of rapidly fermenting manure in 200 c.c. water.

Pringsheim (39) isolated a cellulose-destroying organism which fermented most actively at 55° C. By the addition of volatile antiseptics during the active stage of fermentation, he arrested the growth of the organism and by this means noted the accumulation of glucose and cellobiose in the cultures. He held that this observation proved that these sugars are intermediate products in the fermentation. In a quantitative study of the reaction, he reported a recovery of about 45 per cent. of the cellulose as acetic acid, small amounts of formic acid and large amounts of carbon dioxide and hydrogen.

Langwell and Lynn (40) found active fermentation of sulphite pulp in an 18-hour-old culture inoculated with fermenting horse dung. Khouvine (41) isolated

an organism from human faeces which fermented cellulose over a temperature range of 35 to 51° C., without a distinct optimum. The organism did not grow in an atmosphere where the oxygen tension was higher than 12 mm. Hg. The main products of fermentation were acetic acid, ethyl alcohol, carbon dioxide and small amounts of butyric acid and hydrogen.

The action of thermophilic cellulose fermenters on the fibre of feeding stuffs is being studied by Woodman and Stewart (42) at the present time, with the object of ascertaining whether the reaction may not form the basis of a method for the artificial determination of the digestibility of the fibrous constituent of feeding stuffs, and for following the progress of lignification in growing crops. Encouraging results are being obtained along these lines of enquiry.

It will be noted that in the foregoing studies of bacterial fermentation of cellulose in artificial media, hydrogen, rather than methane, is, apart from carbon dioxide, the main gaseous product. That methane may arise as the main gaseous product, however, is apparent from the fact that the reaction is now employed for the large-scale production of methane from cotton waste and other forms of cellulose. The methane fermentation is undoubtedly the main type of cellulose fermentation in the animal. Indeed, the earlier investigators arrived at the conclusion that the combustible portion of the gaseous products was exclusively methane. That this is not strictly the case is evident from the experiments of Markoff (34), who found that the composition of the gases contained in the paunch of ruminants, as evidenced from the data for 21 samples taken from the paunch of a goat, displayed variation over the following ranges:

CO ₂	14·95 per cent.	to	54·32 per cent.
CH ₄	19·88	"	42·55 "
H ₂	0·05	"	4·07 "

In Markoff's investigations it is noteworthy that an addition of sugar to the paunch content, either through the feed or directly through the fistula, caused no marked change in the amount of free hydrogen. Neither had the addition of large amounts of protein-rich material any noteworthy effect in altering the composition of the gases. On the other hand, however, in the case of fermentation experiments with the paunch contents outside the body, it was found possible to change almost at will the composition of the gases by the addition of varying quantities of sugar. The range of variation in 10 gas samples was as follows:

CO ₂	17·08 per cent.	to	87·49 per cent.
CH ₄	0·12	"	22·80 "
H ₂	0·03	"	20·04 "

The figures show that, outside the animal body, the fermentation processes may be reversed, so that, instead of methane, hydrogen may be produced almost exclusively.

An interesting manifestation of the activities of cellulose-splitting bacteria was noted by Woodman and Hanley (43) in their studies of the changes which occur during the ensilage of a green crop, like rye grass and red clover, in the stack. It

was noted that the temperature of the stack rose abruptly from 18.3 to 71.5° C. in the first two days, and rose further to a maximum of 73.9° C. during the next four days. Between days 6 and 8, quick cooling took place to 62° C. The rate of cooling suffered a check at this stage, the temperature falling only 2° C. during the next four days. Between days 13 and 19, a steady temperature in the region of 60° C. was maintained. Thereafter, a further rise occurred, the temperature curve attaining a second peak of 72.2° C. on day 26. Continuous and fairly uniform cooling took place beyond this point.

The initial rise in temperature to 71.5° C. during the first 48 hours was to be ascribed to the heat engendered by the oxidation of carbohydrate as a consequence of cell respiratory activity, while the second and distinct rise in the temperature of the stack was occasioned by the activity of thermophilic cellulose-splitting micro-organisms. Associated with this behaviour was a very pronounced falling off in the digestibility of the protein in the crop, which fell from 64.5 per cent. in the green crop to 12.2 per cent. in the resulting stack silage. This depression was shown to be due to a portion of the protein in the crop having undergone transformation, during the period of bacterial activity, into a dark brown nitrogenous product, which was insoluble in both hot dilute acid and alkali ($\frac{1}{4}$ per cent. strength) and also was not brought into solution after prolonged treatment with pepsin-HCl at 37° C.

VII. PHYSIOLOGICAL VALUE OF THE PRODUCTS OF THE BACTERIAL FERMENTATION OF CELLULOSE.

Before accepting the theory that cellulose is rendered available to the ruminant organism by bacterial activity, in the rumen, of the nature of that indicated in the preceding section, it will be advisable to consider the nutritive value to the animal of the products of such fermentation. The gaseous products, namely, carbon dioxide, methane and hydrogen, are to be regarded as entirely valueless, since they are excreted as waste products from the body. Indeed, if, as is probable, this breakdown of cellulose is primarily for the purpose of furnishing energy to the micro-organisms concerned, then it would appear that the latter are themselves incapable of utilising the whole of the energy of the cellulose molecule, since methane and hydrogen are oxidisable substances. Of the products of such fermentation, only the organic acids, chiefly acetic acid with traces of butyric acid, and ethyl alcohol can be supposed to be of any value to the animal.

If the theory of the assimilation of cellulose by bacterial fermentation is to be reconciled with the experimental finding of Kellner that digestible cellulose and digestible starch have equal fattening values, then the organic acids resulting from the bacterial fermentation of 1 lb. of digestible cellulose must be equal, for productive purposes, to the glucose produced by the enzymatic digestion of 1 lb. of digestible starch.

What constitutes the actual value of organic acids like acetic acid and butyric acid for productive purposes in the animal is not clear. Little is known respecting the katabolism of the simpler organic acids, beyond the mere fact that they are

oxidised to carbon dioxide and water. Von Knierem (44) has shown that organic acids such as result from the fermentation of carbohydrates are not found to any appreciable extent in the excreta. It may be concluded, therefore, that they are readily absorbed from the digestive tract. Lactic acid and butyric acid, when introduced directly into the circulation of the *fasting* animal in the form of the sodium salts, are promptly oxidised and are able to protect body fat from oxidation (45, 46). It would appear, however, that acetic acid, which is the predominant acid in cellulose fermentation, is inferior to both lactic acid and butyric acid in this respect (47).

Although, as has already been indicated, studies of cellulose fermentation carried on outside the body may by no means give a true picture of what actually goes on, in this respect, in the animal, it is of interest in this connection to consider the results of a quantitative study of the fermentation reaction, between cellulose and thermophilic bacteria, carried out by Viljoen, Fred and Peterson (38). 60 gm. of cellulose were employed in the experiment, of which 42 gm. were actually fermented. The essential results are shown in Table VI:

Table VI. *Fermentation of cellulose by thermophilic bacteria.*

		Carbon balance	
Cellulose fermented	gm. = 42·0	In cellulose	gm. = 18·6
Acetic acid formed*	= 21·6	In acetic acid	= 8·6
Ethyl alcohol ,,"	= 10·3	In ethyl alcohol	= 5·4
Carbon dioxide ,,"	= 11·9	In carbon dioxide	= 3·2
Total products	= 43·8	C. in total products	= 17·2†

* Containing a little butyric acid.

† Carbon deficit due to slight loss of ethyl alcohol under conditions of fermentation.

Complete oxidation of the acetic acid and ethyl alcohol formed in the reaction would yield $(21\cdot6 \times 3\cdot49) + (10\cdot3 \times 7\cdot13) = 148\cdot82$ kg. cals. The thermic energy derivable from the products of fermentation of 1 gm. cellulose is therefore

$$148\cdot82 \div 42 = 3\cdot54 \text{ kg. cals.}$$

This figure would be slightly higher if it were possible to take into account the small amount of butyric acid included in the yield of acetic acid and the slight loss of ethyl alcohol which occurred under the conditions of the experiment.

Kellner (14) has shown, in experiments with oxen, that, for every 100 gm. of starch digested, there is a production of 3·17 gm. methane by fermentation. The heat of combustion of starch is 4·183 kg. cals. per gm.; that for methane is 13·34 kg. cals. It follows, therefore, that the physiological heat value of 1 gm. of digested starch is 4·183 minus $(13\cdot34 \times 0\cdot0317) = 3\cdot76$ kg. cals. The agreement of this figure with that obtained for the heat value of the products formed when 1 gm. of cellulose is fermented in artificial media by thermophilic cellulose-splitting organisms is very striking, and suggests that there should be no difficulty in reconciling

the equal physiological heat values of digestible starch and digestible cellulose on the basis of the accepted theories of digestion of these constituents, namely, that starch is digested mainly to glucose by enzymatic action, suffering a loss of about 10 per cent. of its total energy as a result of methane production by bacterial fermentation, and that cellulose is digested wholly as a consequence of bacterial fermentation to organic acids, mainly acetic acid, and gases, mainly methane and carbon dioxide.

In order, however, that this theory of cellulose digestion shall be brought completely into line with Kellner's finding as to the starch equivalent of digestible cellulose, it is necessary to demonstrate further that the organic acids produced, per lb. of digested cellulose, have a productive (*i.e.* fattening) value equal to the glucose formed by the enzymatic digestion of 1 lb. of starch. Can such organic acids as acetic acid and butyric acid, when fed in conjunction with a ration sufficing for maintenance, lead to the production of fat in the animal? The direct experimental evidence on this point is exceedingly scanty. Kellner⁽¹⁴⁾, with the help of his respiration chamber technique, studied the relation of simple organic acids, such as lactic acid, to fat formation in the body. In an initial period of feeding, he kept a fully-grown wether sheep on a ration containing lactic acid and calcium lactate and measured the amount of flesh and fat formed in the body of the animal. The measurements were repeated in a second period, during which the lactic acid and calcium lactate were omitted from the ration. From the results of this trial, Kellner concluded that the lactic acid had exerted no significant influence on fat formation, but had simply undergone total oxidation in the body with the production of heat. He held that the simple organic acids could only serve as sources of thermic energy in the body.

It may be that too much weight must not be attached to Kellner's result in respect of the inability of lactic acid to undergo conversion into fat in the animal. Lactic acid arises in the organism as an intermediate product of glucose metabolism and as a de-amination product in the metabolism of protein. There is distinct evidence of the power of the animal organism to transform lactic acid into glucose, and presumably, therefore, into fat. If a liver, poor in glycogen, is perfused with blood containing lactic acid, the latter is found to disappear⁽⁴⁸⁾. Glucose is also formed from lactic acid in the phlorizinised dog⁽⁴⁹⁾. Moreover, Lusk⁽⁵⁰⁾ was led to believe that lactic acid and glucose behave similarly in metabolism from the close accord between the specific dynamic actions of glucose and glucose plus lactic acid.

On the other hand, however, the available evidence leaves no doubt that acetic acid is not convertible into glucose in the animal. No glucose is formed from acetic acid in the phlorizinised dog⁽⁵¹⁾. When ingested in admixture with glucose, acetic acid, unlike lactic acid in this respect also, causes a marked increase in the rate of metabolism and heat production⁽⁵⁰⁾. Its rapid oxidation in the body is further indicated by the fact that its ingestion causes no change in the CO₂-combining power of the blood⁽⁵²⁾.

It may be concluded, therefore, that the acids which arise in the bacterial

fermentation of cellulose, namely, acetic acid and traces of butyric acid, are rapidly oxidised in the body and serve only for the production of heat. It might be argued, however, that if the animal derives heat from the oxidation of such acids, then this should spare the oxidation of an isodynamic amount of nutrient in the rest of the ration, thus enabling the energy in this portion to be stored up in the body as fat, instead of undergoing transformation into unproductive heat. That this is not the case, however, is clear from a closer study of the processes involved. Any given ration contains a definite amount of metabolisable energy, the latter being the gross energy of the ration, as calculated on the basis of its heat of combustion, minus those quantities of energy which are eliminated in the gaseous, liquid and solid excreta when the ration is fed to an animal. The metabolisable energy in the ration is not wholly productive. A definite fraction, known as the thermic energy, always undergoes conversion into heat in the animal. This form of energy cannot be re-transformed into the chemical energy of live-weight increase. The remaining fraction, also definite in amount and known as the dynamic or net energy of the ration, is capable of being stored up in the animal in the form of fat or flesh. The addition of easily oxidisable acids, such as acetic acid, to the ration will not alter the amount of dynamic energy available for live-weight increase, since the amounts of thermic and dynamic energy are fixed characteristics of the ration. It will lead merely to increased heat production in the animal, owing to a summation of the thermic energy of the ration and of the added organic acids. The addition of acetic acid simply magnifies heat production without increasing fat production.

It follows that although the organic acids arising from bacterial fermentation of cellulose may, by virtue of their heat value, be useful in the maintenance of bodily warmth and indeed, in the case of the *under-fed* animal, may spare the oxidation of body fat for production of heat, yet they will have no value for fat production in the body of the productively fed animal. The accepted hypothesis that cellulose is rendered available to the animal *solely* by destructive bacterial fermentation to organic acids is not compatible, therefore, with Kellner's finding, that digestible cellulose and digestible starch possess equal values for fattening.

VIII. ALTERNATIVE THEORY OF CELLULOSE DIGESTION BY BACTERIAL AGENCY.

If the theory of cellulose digestion is to be brought into line with Kellner's finding, there appears to be no alternative but to assume that the cellulose complex, under the influence of cellulose-splitting bacteria, undergoes hydrolytic cleavage to glucose, or to a sugar capable of being hydrolysed further to glucose. By such a course of digestion, the cellulose would be assimilated in the form of glucose, and the equal fattening values of digestible starch and digestible cellulose would be capable of simple explanation (53).

If it be assumed that the primary action of cellulose-dissolving bacteria consists in the hydrolysis of cellulose to glucose or some related sugar, there is no reason to assume that the sugar so formed will be broken down further in the rumen to any greater extent than are the "soluble" carbohydrates present in the food originally.

It has already been indicated that, as a result of bacterial fermentation, about 10 per cent. of the energy in the starch of a ration is lost in the form of methane. The amount of such destructive fermentation must be determined by the energy requirements of the micro-organisms. If, then, it can be assumed that sugar arises as the primary product of the bacterial digestion of cellulose, then it may further be assumed that about 10 per cent. of the energy in such sugar is lost by further bacterial breakdown to methane, whilst the remainder is available for utilisation in the organism in the form of glucose. It would follow, therefore, that every lb. of cellulose so digested would yield to the organism as much glucose as would a lb. of digested starch, and by this means it would be possible to explain Kellner's finding.

Woodman (53) has put forward the following scheme of digestion of cellulose and starch to account for the equal productive values of these constituents:

Enzymatic digestion:
Starch* → maltose (glucose- α -glucoside) → glucose.

Bacterial digestion:
Cellulose* → cellobiose (glucose- β -glucoside) → glucose.

* In both cases, about 10 per cent. of the energy is lost as a result of destructive bacterial fermentation to methane.

A striking similarity is assumed in the above scheme between the mode of digestion of starch by enzymes and that of digestion of cellulose by bacteria. The tenability of such a hypothesis, however, depends on the production of evidence to support the view that glucose and cellobiose arise as intermediate products when cultures of cellulose-fermenting bacteria are brought into contact with cellulose in artificial media. This evidence will be brought forward in the final section of this review. A number of other observations may also be cited which lend support to the hypothesis.

Armsby (22) has shown that, on an average, 4.7 gm. of methane are produced in the ruminant tract per 100 gm. of carbohydrate plus fibre digested. This fermentation takes place mainly in the rumen, before the food has come into contact with the enzymatic secretions. In the horse, however, starch and similar carbohydrates are largely or wholly digested by enzymatic action before the food reaches those parts of the digestive tract, namely, the caecum and colon, where it can stagnate and be acted on by bacteria. Consequently, the methane fermentation in this species is substantially limited to the cellulose in the food, the "soluble" carbohydrates, such as starch, contributing little or nothing to methane production. Lehmann, Zuntz and Hagemann (54), on the average of eight experiments with a horse, observed a production of 4.73 gm. of methane and 0.2 gm. of hydrogen per 100 gm. of fibre digested. This figure agrees closely with the amount of methane eliminated in the ruminant per 100 gm. of digested carbohydrate plus fibre, and suggests that cellulose and starch undergo analogous changes during digestion, the same fraction of energy being lost in both cases in the form of methane produced by destructive fermentation.

The amount of methane eliminated by the pig appears to be insignificant.

Fingerling, Kohler and Reinhardt (55) found the amounts of combustible gases excreted to be too small to be determined in their form of Pettenkofer apparatus. Von der Heide and Klein (56), in experiments with a Regnault-Reiset apparatus, obtained the following average for three pigs: 0·65 gm. methane and 0·07 gm. hydrogen eliminated per 100 gm. digested carbohydrate plus fibre. This small production of methane may, of course, merely reflect a low assimilation of cellulose from the experimental rations. On the other hand, however, it may signify that digestion of cellulose by destructive bacterial fermentation to organic acids and methane fails to account for the power of swine to utilise, very efficiently, the fibre of non-lignified feeding stuffs. A repetition of the experiments, using a feeding stuff rich in non-lignified cellulose, such as sugar beet pulp, might yield data of a critical nature in respect of the question under consideration.

Kellner (14) has demonstrated that when starch, cellulose and cane sugar are separately added to the maintenance rations of bullocks, the portions of the energy, contained in the digestible fractions of these constituents, which are stored up as fat in the animal are 56·4, 57 and 45·2 per cent. respectively. The digested starch and cellulose exert, therefore, an almost equal productive effect. If cellulose were fermented more completely than starch, with the production of a larger proportion of useless gaseous products, then a smaller percentage of its potential energy would be found in the live-weight increase. That this would be so is shown by the lower productive value of cane sugar, which, on account of its solubility and simpler chemical constitution, is more readily attacked and broken down by the fermentative micro-organisms than the more stable and complex polysaccharides. It will be noted that the fraction of the energy of the sugar which is stored in the live-weight increase is distinctly lower than is the case with cellulose and starch.

IX. THE TRANSFORMATION OF CELLULOSE INTO GLUCOSE BY THE AGENCY OF THERMOPHILIC CELLULOSE-SPLITTING MICRO-ORGANISMS.

The theory of bacterial digestion of cellulose outlined in the foregoing section rests on the assumption that glucose arises as an intermediate product when cultures of cellulose-fermenting bacteria are brought into contact with cellulose in artificial media. Evidence in support of this contention has been provided by the investigations of Pringsheim (39) and of Woodman and Stewart (57). The latter investigated the activity of a culture of thermophilic cellulose-splitting micro-organisms, obtained by incubating actively fermenting horse dung in a medium containing, per 100 c.c., 2 gm. pulped filter paper, 1·2 gm. calcium carbonate, 0·5 gm. sodium phosphate, 0·25 gm. ammonium sulphate and 0·1 gm. potassium chloride. The organism appeared to grow satisfactorily in the absence of organic nitrogen. After making the 106th sub-culture, however, it began to display a rapidly waning power to attack cellulose, and the discovery was made at this stage that the addition to the medium of a little organic nitrogen, in the form of casein, had the effect of renewing the fermenting power of the culture.

In numerous experiments Woodman and Stewart demonstrated the possibility

of converting cellulose into glucose by controlling the activity of the cellulose-fermenting bacteria. The procedure adopted in these experiments was to allow the fermentation of filter paper (or of fibre prepared from oat and tare silage) to proceed unchecked, at 65° C., until gas evolution had become so considerable, that the bulk of the fermenting material was carried to the surface of the liquid, forming a "head." The time for this stage to be reached was from 2 to 3 days. While the fermentation was at its height, toluene was added with shaking, and the incubation was continued at 37° C. for about 5 days, after which there was no difficulty in demonstrating the presence of glucose in the reaction mixture.

At the stage of fermentation when toluene was added, no glucose was present in the medium. As the addition of toluene inhibited the further activity of living organisms, it was concluded that the glucose which arose during the second stage of the fermentation was the result of the activity of enzymes which had been elaborated and secreted by the organisms during the first stage of the fermentation.

It appears reasonable to assume, therefore, that cellulose-splitting bacteria are unable to utilise directly, for metabolic purposes, the complex cellulose molecule, but must first cause this to be hydrolysed to the simpler form of glucose. This purpose is achieved by the agency of a cyto-hydrolytic enzyme and, possibly, of other enzymes also, such as cellobiase. Here we appear to be dealing with a process of digestion comparable with that associated with higher organisms. Under normal conditions of fermentation, the glucose so formed would readily be assimilated and utilised by the micro-organisms for metabolic purposes, the end products of such metabolism being organic acids, methane, carbon dioxide and traces of hydrogen. The addition of toluene, however, at the "head" stage of fermentation, rules out the possibility of metabolic activity of this character, so that any glucose formed in the second stage of the reaction, by the continued activity of enzymes, is enabled to escape destruction.

The yield of glucose, under the conditions of these experiments, was quite small. In one experiment, for example, starting with 40 gm. of pulped filter paper, 4.2 gm. of a caramel-like, glucose-rich substance was isolated from the reaction mixture. This, however, does not preclude the possibility of discovering conditions under which the yield of glucose might be increased considerably, in which case the reaction might conceivably acquire a technical significance, as, for instance, in the production of power alcohol.

No further destruction of filter paper appears to take place in the second stage of fermentation after the addition of toluene. It is probable, therefore, that the enzymes present in the second stage are concerned in producing glucose from partially hydrolysed cellulose, and that the primary cyto-hydrolytic process is dependent on the presence of the living organisms. This hypothesis, which would explain the low yields of glucose obtained in the reaction, would further account for the negative results, in respect of fermentative activity, which were obtained when filter paper was incubated with the liquid resulting from filtering a culture of the organisms at the "head" stage through a bacterial candle.

Continued sub-culturing of the micro-organism in the absence of cellulose

leads to a loss of its power to ferment cellulose. It is probable that this behaviour is to be ascribed to a gradual atrophy of the function which enables the organism, under normal circumstances, to produce glucose as a primary product from cellulose. If this be so, support is given to the view that such cellulose-fermenting bacteria possess a dual mechanism, whereby, firstly, they hydrolyse cellulose to glucose, and secondly, ferment the glucose to organic acids and gases.

Although it has been demonstrated that when the fermentation of cellulose by bacteria is controlled by the addition of toluene at the "head" stage, this is followed by the appearance of small amounts of glucose, the main question remains to be settled, however, as to the nature of the factor which controls the bacterial action on cellulose in the rumen of the animal. Here the control is so efficient that something like 90 per cent. of the energy of the digestible cellulose becomes available to the animal in the form of glucose. Surprise may be felt that so little of the glucose formed by bacterial action should undergo destructive breakdown to methane; yet this is scarcely more surprising than that only about 10 per cent. of the energy of the digestible carbohydrate (*e.g.* starch) *originally* present in the food should be lost as methane arising from destructive fermentation.

Further investigation will be necessary before the changes which occur in the rumen of the animal can receive adequate explanation. It may be that the extent of destructive fermentation of both carbohydrate and cellulose is determined by the energy requirements of the micro-organisms. If, however, the primary conversion of cellulose into glucose is brought about by enzymes elaborated by the bacteria, there is no reason why this hydrolysis should be so regulated as to produce just sufficient, and no more, glucose as will satisfy the metabolic requirements of the micro-organisms. Bearing in mind the catalytic properties of enzymes, and their capacity for carrying out their functions independently of the living organism, it is not unreasonable to picture a continuous production of glucose from cellulose in the rumen quite irrespective of the limited requirements of the micro-organisms, which may be regarded solely, in this connection, as elaborators of the requisite enzymes.

Although the experiments referred to in this section were carried out with micro-organisms derived from fermenting dung, it should be pointed out that a culture of bacteria, possessing the same powers and characteristics, has also been obtained from the contents of the sheep's rumen⁽⁴²⁾. It is, however, difficult to understand why these cultures, when investigated in artificial media, require a temperature in the neighbourhood of 65° C. for the marked display of their fermentative activity, although, in the animal, the same activity must be going on at the low temperature of 37° C. It must be concluded, therefore, that although Kellner's work on the fattening value of digestible cellulose in the ruminant animal leads to the belief that glucose is formed from cellulose in the rumen on an almost quantitative scale, the hypothesis put forward in this review, as to how this occurs, must remain speculative until more supporting evidence has been obtained from work in artificial media than has been accumulated up to the present.

X. SUMMARY.

The question of the rôle which cellulose plays in nutrition is of particular importance to the agriculturist, since fibre constitutes a very significant part of the normal rations of farm animals. The fibre in plants and feeding stuffs consists of cellulose, admixed with varying amounts of lignocellulose. In the young plant, the cell walls are composed almost wholly of cellulose. The latter is digested in the ruminant tract almost to the same extent as is the carbohydrate component. As the plant matures, however, the cell walls become toughened by the deposition of the encrusting substance, lignocellulose. The latter is entirely indigestible. The process of lignification is accompanied, therefore, by a diminution in the digestibility of the fibre of the crop. In other words, the digestibility of fibre is inversely proportional to its content of lignocellulose. For reasons made clear in the text, the digestibility of the other food nutrients, such as protein, oil and carbohydrate, also displays a corresponding running-off during lignification.

Ruminant animals have the greatest power of digesting the fibre in feeding stuffs. Although swine are able to assimilate the cellulose of non-lignified feeding stuffs almost as completely as can sheep and cattle, they are unable to make such good use of lignified fibre. Horses can digest the fibre in their rations, but not with the degree of efficiency displayed by ruminants. In the human alimentary tract, the less resistant forms of cellulose are digested quite readily, while the power of certain invertebrates to subsist on cellulose is very marked. Birds are able to utilise the fibre in their food to a limited extent.

Our knowledge of the value of cellulose in the nutrition of farm animals is derived from the results of Kellner's investigations. Kellner has shown that if 1 lb. of pure, finely divided cellulose be added to the maintenance ration of a bullock, as much fat, namely $\frac{1}{4}$ lb., will be formed in the body of the animal as would be produced by the addition of 1 lb. of starch or other digestible polysaccharide. The starch equivalent of digestible cellulose is unity. Under ordinary conditions of feeding, however, when the cellulose is embedded in indigestible fibre, the net starch equivalent derived from 1 lb. of digestible fibre is less than unity, since a deduction must be made on account of the energy used up by the animal in masticating the indigestible portion and in separating the cellulose from the incrusta.

The presence of cyto-hydrolytic enzymes has been demonstrated in seeds and other plant tissues, in the hepato-pancreatic secretions of several species of land snails and in the saliva of certain insects. The breakdown of cellulose in the digestive tracts of higher animals, however, is not the work of a cytase present in any of the digestive secretions, although it is conceivable that the cytases present in certain types of plant food may become active under the favourable conditions provided by the alimentary canal. Digestion of cellulose in the animal tract is mainly brought about by the fermentative activity of cellulose-splitting bacteria, the digestion taking place in the parts of the tract where the food stagnates, as, for example, the rumen in sheep and cattle, and the caecum and colon in the case of horses and pigs.

Digestion of cellulose by enzymes gives rise to the sugars, cellobiose and glucose.

The bacterial fermentation of cellulose in artificial media, on the other hand, gives rise to organic acids, mainly acetic acid and a small amount of butyric acid, and gaseous products, such as carbon dioxide and either methane or hydrogen. Ethyl alcohol may also be formed in the fermentation. Experiments carried out on ruminants in respiration chambers have demonstrated that the methane fermentation is the main cellulose fermentation which occurs in the animal. A consideration of the physiological value of these breakdown products, however, leads to the following conclusion: Although the organic acids arising from bacterial fermentation of cellulose may, by virtue of their heat value, be useful in the maintenance of bodily warmth, and indeed, in the case of the *under-fed* animal, may spare the oxidation of body fat for production of heat, yet they will have no value for fat production in the body of the productively fed animal. The accepted hypothesis that cellulose is rendered available to the animal *solely* by destructive bacterial fermentation is not compatible, therefore, with Kellner's finding that digestible cellulose and digestible starch possess equal values for fattening.

The following scheme of digestion of cellulose and starch is put forward to account for the equal productive values of these constituents:

Enzymatic digestion:



Bacterial digestion:



* In both cases, about 10 per cent. of the energy is lost as a result of destructive bacterial fermentation to methane.

Evidence in support of this theory of bacterial digestion of cellulose to glucose in the animal is provided by the results of recent work on the conversion of cellulose into glucose, in artificial media, by controlling the activity of cellulose-splitting micro-organisms. The full significance of this evidence is discussed in the text.

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RESULTS OF SOME RECENT STATISTICAL INVESTIGATIONS OF INVERTEBRATE FOSSILS

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(Received June 6, 1930.)

(With Nine Text-figures.)

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I. INTRODUCTION.

THE statistical investigations of the Chalk Micrasters by Rowe (1899) produced results which were alike of value to the stratigraphical geologist and of interest to the biologist. An account will be given here of the results of some more recent studies of similar character, chiefly relating to various groups of Mollusca. Such Invertebrates frequently afford satisfactory material for studies of this kind, since they occur in great numbers at many horizons; similar studies of fossil Vertebrates, if they could be made, would perhaps be of greater interest on account of the larger number of characters which are available for study, but it is rarely possible to collect adequate material from any bed at one locality. In the case of certain Mollusca, it is possible to collect in a layer a few inches thick some hundreds of specimens of one species within a space of no more than a few square yards. As such specimens may represent only a few generations and as, in the case of many Mollusca, they are the remains of more or less sedentary forms, which lived where they are now found, it is perhaps legitimate to regard such a collection as typical of a freely interbreeding community.

It may, of course, be admitted that those who study fossil shells are concerned wholly with the skeletal parts of the animal, and are only able to obtain limited information as to the characters of those soft parts which are intimately related to the skeleton. The palaeontologist, however, has necessarily to be content with this limited material, and although it is incomplete it is suggested that it yields results of some biological interest.

II. THE LINEAGE OF *GRYPHAEA INCURVA*.

In the case of *Gryphaea*, which has been dealt with elsewhere (Trueman, 1922), it is possible to obtain great numbers of specimens at many horizons and in many localities.

It has been shown by Dr F. L. Kitchin (1912) that, repeatedly during the Mesozoic, oyster-like forms became progressively modified into the forms known as *Gryphaea* (Kitchin, 1912; see also Lang, 1923). This change resulted from a reduction of the area by which the left valve was attached, by a thickening of that valve, and by that valve becoming incurved. Thus, in later members of the series, the left valve of the shell was completely turned through a whole whorl, while the right valve remained practically unaltered and flat. In some of the examples the curvature of the shells is so extreme that the left valve presses against the right, and thus leads to the death of the animal and to the extinction of that line of evolution. This set of changes from an ordinary oyster-like shell to *Gryphaea* may be traced several times in the Jurassic; each Gryphaean offshoot is thus unconnected with those that preceded it, and represents an independent development from what may have been (at any rate in part) a common ancestral stock.

Attention has chiefly been paid to the shells which lead up to the form known as *Gryphaea incurva*. This species occurs very commonly in the Lower Lias, in the upper part of the Bucklandi Zone (Trueman, 1922). It has been shown that a practically continuous series of forms can be found linking the flat oysters of the Planorbis Zone with the completely incurved Gryphaeas. Indeed, it is not likely that there are many lineages in which so considerable and obvious changes can be more easily demonstrated. In Glamorgan, where these zones of the Lias are most thickly developed and continuously exposed, these changes are found to have taken place while less than 300 feet of strata were deposited, and it may be suggested that, as compared with Lamellibranchs in general, the evolution of the Gryphaean form was relatively rapid.

While a continuous series of intermediate forms representing every stage from *Ostrea* to *Gryphaea* may be found in these zones, it has been shown that at any one horizon there is considerable variation in the degree of curvature in the forms which are present (Trueman, 1922, pp. 262-263). Thus, in any bed, a study of the amount of curvature of the left valve in the adult forms shows that the majority of the specimens conform to one type, but that certain proportions are respectively more advanced and less advanced (in respect of coiling) than the mean of the group in that bed. Plotting the variation among the specimens, which may be considered as representing one community, it is found that they constitute a single and homogeneous group, and that they include forms which vary continuously, linking by imperceptible gradations the extreme forms at that horizon.

At higher horizons the variation is similar in range and character, but the mean type is more incurved, the least advanced forms are more curved than the least advanced forms of lower horizons, and the most advanced are more curved than the most advanced of lower horizons.

The evolution of *Zaphrentis delanouei*, investigated by R. G. Carruthers (1910), appears to afford similar evidence of a group showing a steady progressive change in a given direction, the group being represented on each horizon by forms which differ among themselves in the degree of advancement attained in certain features. Contemporary members of a community differed among themselves in just those characters which distinguished the normal forms of one community from the normal forms of earlier or later communities.

Various problems arise in relation to these instances, which may usefully be dealt with before considering other cases. In the first place, it may be emphasised that there is apparently continuous variation in the skeletal characters among the forms at one horizon, and a similar continuous change in time. It follows from this, in the case of *G. incurva* at least, that there can be no satisfactory grouping of the various forms into species. For, although the forms at any one horizon constitute a natural and apparently homogeneous group, such as would be regarded as a single species, the separation of one species from another is impossible when the whole group is considered, except on admittedly arbitrary lines. It may be useful to express this diagrammatically. Suppose below that three successive horizons with *Gryphaea* are represented. At the lowest horizon, the forms present may be considered

Horizon (iii)	27	28	29	30	31	32
„ (ii)	26	27	28	29	30	31
„ (i)	25	26	27	28	29	30

to have attained stages in evolution represented by the numbers 25 to 30; it will of course be noted that a great proportion of the community will be at stages 27 and 28, but this fact need not detain us here, for it is with the occurrence of certain forms, and not their abundance, that the present discussion is concerned. Now if it were decided that all the forms in the lower horizon (i) constitute one species, there is no reason why the forms at horizons (ii) and (iii) should not equally be considered species. But since some of the forms are identical with some of the forms in horizon (iii), it is not possible to effect such a separation unless the date (or horizon) is included as a factor. Moreover, if an arbitrary line is chosen, such as that between stages 30 and 31, then this line will, at some point, cut across the members of a presumably interrelated community (and may conceivably separate the offspring of the same parent).

These problems arise at every stage in such a line of evolution, and if a continuous series is available, there is no satisfactory line which can be drawn to separate the flat oyster from the latest forms of *G. incurva*. This might lead to placing the whole group in a single species. Dr W. B. Crow (1926), in discussing this, suggested that similar instances occur in the Blue-Green Algae, where there are innumerable intermediate links between the accepted species. It may be pointed out, however, that it is not the occurrence only of intermediate links in one community which is emphasised here, but the fact that a statistical examination of the community reveals that, in respect of the characters which can be examined, it is a homogeneous group; there are not, as appears to be the case in the instances quoted by Crow,

several well-marked types connected by a smaller proportion of intermediates, but considering one community of *Gryphaea*, the widely differing extremes are linked by intermediate forms which constitute the main part of the community.

Crow has further suggested (1926, pp. 122-123) that the various members of a community of *Gryphaeas* may have been derived from the same parent stock on more than one occasion. This would imply, apparently, that the group of *G. incurva* consists of a number of parallel lines of evolution. In other words, referring to the diagram above, the form 25 in horizon (i) has given rise to form 26 at horizon (ii), and form 27 at horizon (ii) to that numbered 28 at horizon (iii), etc.; the first-named having been derived (according to Crow's suggestion) from the parent stock at a later stage than the associated forms.

It is difficult to accept such an interpretation in view of the fact that the proportions of the individuals at the various stages are constant at a horizon over wide areas, and that, so far as has been ascertained, the variants are definitely related to a mean type. It appears therefore that the group of *G. incurva* does not consist so much of a bundle of parallel lineages as of a series of intermingling or anastomosing lines. This conception will be discussed further below.

Crow suggests that the production of almost identical forms (that is, of homoeomorphs), particularly in the Ammonites, to some extent illustrates his point of view, but it may be noted that such homoeomorphs are frequently known to have been derived from unrelated stocks and often at long intervals of time. Such instances may in some cases be compared with the evolution of successive series of *Gryphaea* from flat-shelled oysters at various times during the Jurassic, but they cannot be considered as comparable with the variants in a single community.

III. VARIATION IN COAL MEASURES LAMELLIBRANCHS.

In recent years considerable attention has been paid to the Lamellibranchs which occur commonly in the Coal Measures, and which have been referred to the genera *Carbonicola*, *Anthracomya* and *Naiadites*. These shells are now generally regarded as having been non-marine forms, possibly fresh-water or estuarine. It may be suggested that there is a superficial similarity between *Carbonicola* and *Unio*, and between some species of *Anthracomya* and *Anodonta*. Possibly they lived under somewhat similar conditions. Like many modern sedentary molluscs, these shells exhibit a great deal of variation and, partly for this reason, they have been long neglected by stratigraphers, both in the zoning of Coal Measures and in the identification of coal seams. Extensive studies of the variations in these shells have been carried out, chiefly with a view to their being used for these purposes. The most important statistics have related to the genus *Carbonicola*, which is more abundant in many beds than the other genera. A typical example of such an investigation is illustrated in Figs. 1 and 2. The specimens were collected above the Lower Vein at Brynamman, South Wales (Davies and Trueman, 1927, p. 210). Measurements were made of the length of the shell (from anterior end to posterior end, parallel to the hinge line), the greatest height (at right angles to the measured length), the length of the anterior end and the thickness (or tumidity). The last

three dimensions were expressed as percentages of the length. The shells were also grouped according to the form of the lower border into four classes (strongly convex, slightly convex, straight, and concave).

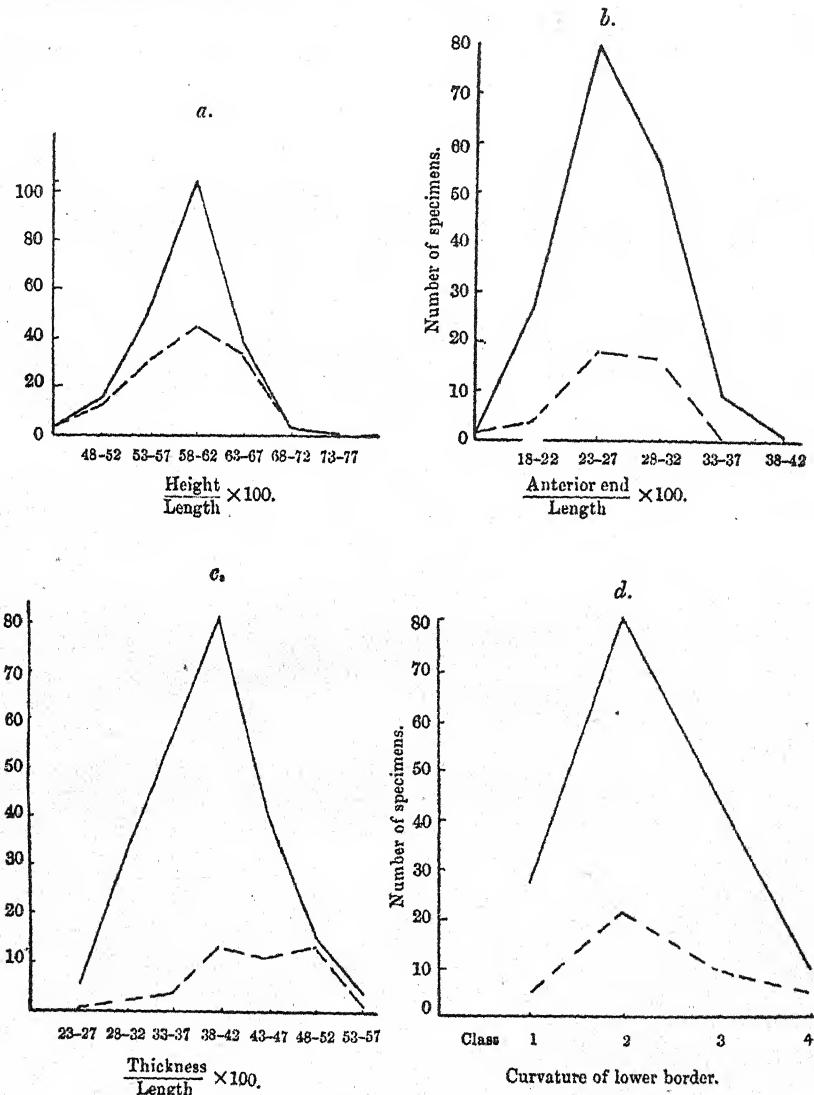


Fig. 1. Graphs illustrating the variation in communities of *Carbonicola* aff. *communis* in the Brynamman district (continuous lines) and at Bonville's Court, Pembrokeshire (broken lines). After J. H. Davies and A. E. Trueman. [Reproduced from the *Quarterly Journal of the Geological Society*, 1927, 83, p. 216, Fig. 3, by permission of the Council.]

It will be noted that, in each of these characters, there is wide variation in this community (Fig. 1). For example, there are extremely elongated shells with height only 45 per cent. of the length, which are linked by numerous intermediate forms

with shells having a height equal to over 70 per cent. of the length. Although the range of variation is so considerable, however, the community appears to be homogeneous so far as is shown by the characters examined, and to be grouped fairly symmetrically around a mean type.

The relations of the variations in these characters were further studied by means of scatter-diagrams (Fig. 2). Briefly, it may be pointed out that little correlation

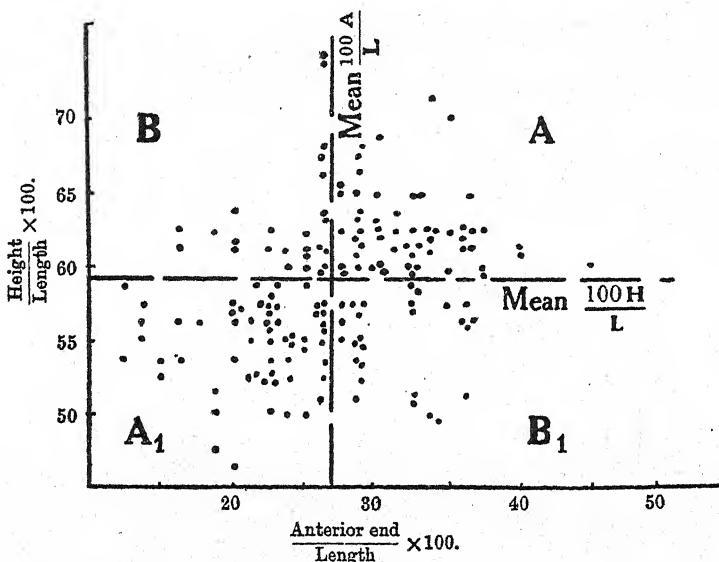


Fig. 2. Scatter-diagram illustrating the variation in a community of *Carbonicola* aff. *communis* from the Brynamman district. After J. H. Davies and A. E. Trueman. [Reproduced from the *Quarterly Journal of the Geological Society*, 1927, 83, 217, Fig. 46, by permission of the Council.]

was found to exist between the length of the anterior end and the height (both expressed as percentages of the length). Calculated by the Bravais-Pearson product moment formula, the coefficient of correlation of height/length and anterior end/length was found to be 0.28. Similarly there was found to be little correlation between any pair of variable characters, all of which appear to vary to a large extent independently of other characters. There is, of course, a greater degree of correlation between the ratio height/length and the form of the lower border, the forms with a convex lower border being generally higher in proportion than those with a concave border.

Similar studies have been made, primarily for comparison for stratigraphical purposes, in communities at the same and other horizons in other areas. Thus, in Fig. 1, the broken line in the graphs represents a community from above the seam which is equivalent to the Lower Vein in Pembrokeshire (nearly 40 miles west of Brynamman). It will be noted that these shells show variation similar in extent and character to that in the contemporaneous and related community, except in regard to thickness of the shell, a feature which has been found to be most variable in several areas (Davies and Trueman, 1927, p. 215).

In Nottinghamshire and Derbyshire an almost contemporaneous community gave practically the same results (Clift and Trueman, 1929, p. 77), except for differences due to the greater size of the shells examined, the larger shells tending to have a greater height in proportion to length. In that coalfield also an examination of several communities from different localities but at the same horizon showed that there was similar variation in each community.

IV. VARIATION IN SOME FOSSIL GASTROPODS.

Recently, A. Stuart (1927) and T. H. Rowlands (1928) have carried out investigations in Tertiary Gastropods from the Paris Basin. In these studies it has been found possible to pay attention to the variation in the development of the ornamentation, as well as to variation in shell form.

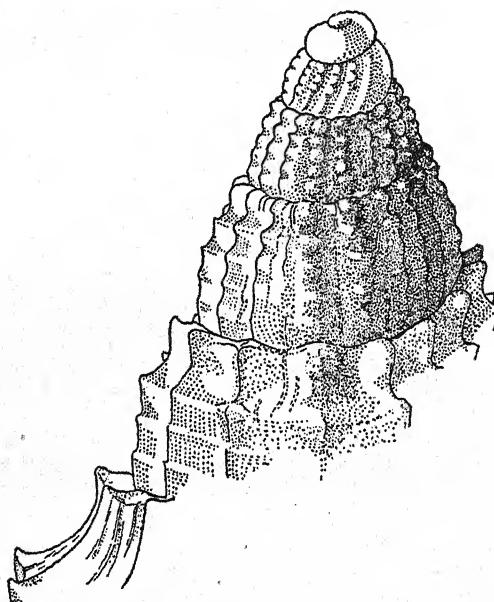


Fig. 3. Ideal development of ornamentation in *Volutospina spinosa* (Linn.) $\times 20$. After A. Stuart. [Reproduced from the *Geological Magazine*, 1927, 64, 552, Fig. 7, by permission of the editor and of the author.]

In the case of *Volutospina spinosa* (Linn.) Stuart was able to secure 132 shells from one horizon in the Eocene at one locality, the majority of these being sufficiently well-preserved to give details of the development of the ornament from the protoconch onwards. The variation in shell shape was found to be slight, but the breadth/height ratio was found to increase with the age of the individual, and the spire was proportionally longer in young individuals.

The variations in the development were studied in great detail, the appearance of the various elements in the ornamentation being recorded to the nearest one-tenth of a volution. The shells are typically ornamented by costae bearing sharp

spines (Fig. 3). In development, a smooth protoconch is followed by neponic whorls bearing at first simple axial ribs, tubercles appearing on these very soon afterwards. A single tubercle is frequently present on the second rib, a further tubercle (situated below the first) appearing usually on the third rib and occasionally even simultaneously with the first, on the second rib. By the time the eighth costa has appeared, there are usually five rows of almost equal tubercles. After a short time the two upper tubercles become more prominent and the space between

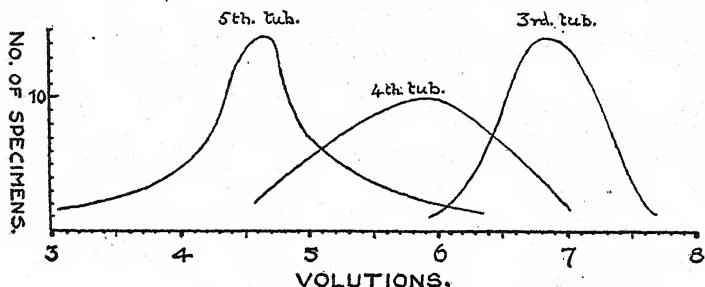


Fig. 4. Curves showing the variation in the stage at which the third, fourth and fifth tubercles disappear in *Volutospina spinosa* (Linn.). After A. Stuart. [Reproduced from the *Geological Magazine*, 1927, 64, 554, Fig. 10, by permission of the editor and of the author.]

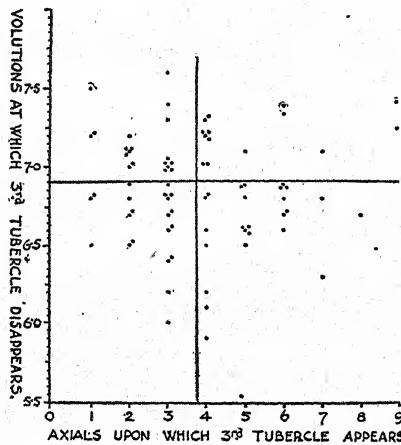


Fig. 5. Scatter-diagram showing the relation between the appearance and disappearance of the third tubercle in *Volutospina spinosa* (Linn.). After A. Stuart. [Reproduced from the *Geological Magazine*, 1927, 64, 553, Fig. 8, by permission of the editor and of the author.]

them increases, while the three lower tubercles disappear. There is marked variation both in the time of appearance and of disappearance of these three tubercles, the fifth tubercle showing the greatest variation in this character. Thus it may appear as early as the third costa, or not until the twentieth, while it may disappear on the third volution or be retained until the sixth volution. The range in the variation in the disappearance of the third, fourth and fifth tubercles is shown diagrammatically in Fig. 4. It is also noteworthy that there is little correlation between the

time of appearance and the time of disappearance of any tubercle in the same individual; this may be seen from the scatter-diagrams, Figs. 5 and 6. Stuart has determined the coefficient of correlation between the position of appearance and the position of disappearance of the fifth tubercle as 0.144.

Similarly, in regard to the other characters studied in that species, the variations in one character are mainly independent of those in another.

In *V. spinosa* it appears that the latitude of variation is not so great as to lead to very notable differences in the adult shells, and only one form, *V. triplicata* (Linn.), which has received a specific name, is regarded by Stuart as falling within the limits of the variations described for *V. spinosa*. In the case of certain other Gastropods, the range of variation is much greater, and members of a single community differ so markedly that they might well be regarded as distinct species if they were not clearly members of one homogeneous "species-group." This was shown to be the

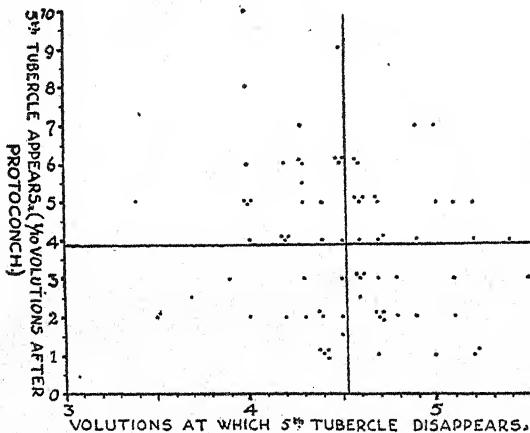


Fig. 6. Scatter-diagram showing the relation between the appearance and disappearance of the fifth tubercle in *Volutospina spinosa* (Linn.). After A. Stuart. [Reproduced from the *Geological Magazine*, 1927, 64, 553, Fig. 9, by permission of the editor and of the author.]

case in *Planorbis multiformis* by Prof. G. Hickling (1913), and is also true of the forms associated with *Katopatra obliquestriata* Trueman (1924 a), and of the community of *Batillaria pleurotomoides* (Lam.), investigated by Mr T. H. Rowlands.

B. pleurotomoides is a Cerithium-like form, of which a large suite of specimens have been studied from the Eocene of the Paris Basin. Some 240 specimens gave information as to ontogeny, and a careful record was made of the development of ornamentation in these forms. Typically, the smooth early whorls are succeeded by the nepionic volutions with spiral lines only, of which three are strongly marked. On about the fifth whorl these spirals are crossed by costae, a small tubercle being present on each rib where it crosses the spiral. Thus in many specimens a tri-tuberculate stage is attained. Later, the anterior row of tubercles disappears, and frequently on about the twelfth volution the next row disappears, leaving only a single row. In a few shells this also disappears, leaving the last whorl almost smooth.

There is some variation in the number of whorls with spirals only (that is, in the stage at which the earliest costae appear), as shown in Fig. 8. In occasional specimens the stage with spirals only is omitted, while in other specimens it extends over seven volutions. There is thus great variation in the stage at which three tubercles appear; on the average the tri-tuberculate stage persists for about five volutions, but it may extend in some cases for only one or two whorls, or in other cases for nine whorls (Fig. 9).

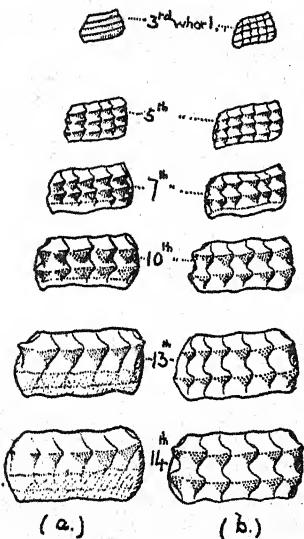


Fig. 7. Development of ornament in *Batillaria pleurotomoides* (Lam.). (a) shows normal development of ornament; (b) shows retardation of the two-tuber stage. After T. H. Rowlands. [Reproduced from the *Geological Magazine*, 1928, 65, 538, Fig. 12, by permission of the editor and of the author.]

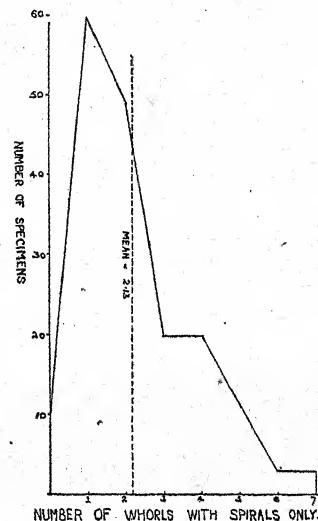


Fig. 8. Variation in the number of whorls bearing spirals only, in the development of *Batillaria pleurotomoides* (Lam.). After T. H. Rowlands. [Reproduced from the *Geological Magazine*, 1928, 65, 532, Fig. 1, by permission of the editor and of the author.]

Similarly, the second row of tubercles may disappear as early as the eighth whorl, or it may persist until the sixteenth whorl. In 53 per cent. of the specimens it disappeared on the thirteenth or fourteenth whorl.

Rowlands has also attempted to ascertain the degree of correlation between the appearance and disappearance of the various elements in the ornamentation. Briefly, it may be noted that he obtained evidence of only low correlation between each set of variants. In his study of the strength of the ornament (considered by reference to a selected set of specimens) he found that those shells which acquire costae early in ontogeny have a slight tendency to develop stronger ornament, but the coefficient of correlation which he determined was only 0.201.

V. THE CONCEPTS OF LINEAGE AND SPECIES.

It will now be useful to extend the consideration of the nature of a lineage already introduced in the discussion of *G. incurva*. It was there suggested that that group might be regarded as consisting of a bundle of anastomosing rather than parallel lines. The other examples described above appear to lend strong support to such a contention. For in each of these cases the variations examined are in more than one set of characters, and, on the whole, variation in one character is independent of variation in another. It therefore follows that a group, such as that of *B. pleurotomoides*, cannot be regarded as a bundle of parallel lines, unless there are an infinite number of such lines, representing almost every conceivable combination of the variable characters. It is difficult to express this case except in graphical manner. Supposing the group is progressing in three sets of characters, *A*, *B*, and *C*, in each

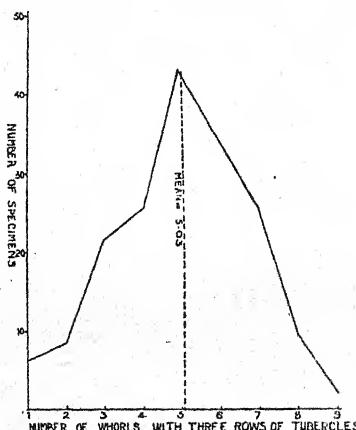


Fig. 9. Variation in the number of whorls on which three rows of tubercles persist in the development of *Batillaria pleurotomoides* (Lam.). After T. H. Rowlands. [Reproduced from the *Geological Magazine*, 1928, 65, 532, Fig. 3, by permission of the editor and of the author.]

of which variation is to a large extent independent, and that the stages of progression are numbered as was done above in the case of *G. incurva*. Then, at any horizon, one individual may have attained stage 30 in character *A*, stage 32 in character *B*, and stage 31 in character *C*; such an individual may be recorded as *A* 30, *B* 32, *C* 31. But, since variation in each of these characters is to a large extent independent, other individuals in the community may include *A* 25, *B* 30, *C* 35; *A* 26, *B* 28, *C* 30; *A* 35, *B* 30, *C* 26; *A* 27, *B* 26, *C* 30, and many other combinations. It will then be realised how complex the group must be if *A* 25, *B* 30, *C* 35 can only interbreed with individuals having a similar index, and not with other members of the community.

It may, of course, be objected that, in the cases of *B. pleurotomoides* and *V. spinosa*, communities from only one horizon have been considered, and that at present nothing precise is known of the earlier and subsequent evolutionary history of the group. These objections do not appear seriously to affect the present discussion.

The ornament features which have been discussed have been acquired by progressive stages, and the differences observed result from the acceleration or retardation of the several features. It does not affect the problem whether the group is at that stage progressing or retrogressing, for the general constitution of the group would be similar in either case.

It thus appears reasonable to consider a lineage as a plexus of anastomosing lines rather than as a single line or a bundle of such lines. The breadth of the plexus may be great or small: it may be so small that, for all practical purposes, the palaeontologist is justified in regarding it as a single line, or it may be so great that the lineage must be studied statistically if the forms comprising it are to be used for stratigraphical purposes.

The difficulty of recognising distinct species within such a lineage will be apparent. If the lineage is incompletely known, owing to gaps in the geological record, the species may correspond to some natural and homogeneous group, but if the conception of lineage put forward here represents a correct view of the facts, in such cases it is clearly impossible to distinguish species. It may be urged, as G. C. Robson has suggested, that some groups (many of which have been studied by the palaeontologists), such as *Zaphrentis delanouei* and *G. incurva*, may be highly susceptible to environmental influences, and "may give the impression of very imperfect differentiation into species, while others, less susceptible and in a more stable physiological condition, may maintain specific individuality" (Robson, 1928, p. 46). It is not unlikely that the group of *G. incurva* and many species of non-marine Lamellibranchs are to be interpreted as peculiarly susceptible to such influences and, therefore, that the results of a study of such examples may be to some extent misleading, if they are not interpreted in relation to the evidence derived from more stable forms. But, although the variation recorded in *Carbonicola* may be much wider than that in other forms, notably in *V. spinosa*, it appears that the variation in the latter is otherwise similar in character. The practical problems of classification in such cases may not be so difficult, but the actual constitution of a lineage may be just as complex.

It has been pointed out that, at any plane in time, a lineage appears to be represented by forms which vary among themselves in ways which recall the differences occurring in the "impure species" of biologists (Trueman, 1924 b, p. 360). Nevertheless, one important distinction has been made, for whereas Jordanons generally differ from one another in sharply contrasted characters, the variations in the characters in members of such groups as are described above merge into one another by imperceptible gradations. The relations of these characters to those which Mendelian analysis shows to be carried by a single gametic factor, is a problem on which little information is available (Swinnerton, 1921; Bather, 1927, p. lxii; 1928).

VI. SUMMARY.

Statistical investigations of Lamellibranchs and Gastropods show that there is considerable variation in any series collected at one horizon. The variation is greatest in such sedentary forms as *Gryphaea* and *Carbonicola*, but the variations

appear to be similar in character, although they are not so wide in range, in certain Gastropods.

At each horizon variation appears to be continuous, and the characters, on the whole, vary independently. Each community is apparently homogeneous and indivisible. It is suggested that such an evolving stock must be regarded as a "plexus" or bundle of anastomosing lineages.

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DIE OSMOREGULATION WASSERLEBENDER TIERE

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(Received June 18, 1930.)

(With Nine Text-figures.)

GLIEDERUNG

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I. EINLEITUNG.

Die Körperflüssigkeit, oder um einen Ausdruck Cl. Bernards zu gebrauchen, "das innere Medium" der wasserlebenden Organismen steht in dauernder Beziehung zu dem umgebenden Medium. Einmal sind die an das Aussenmedium angrenzenden Membranen der Wassertiere fast stets für Wasser durchlässig, dann können wir aber auch bei ihnen, ebenso wie bei den Landtieren, einen dauernden Wasserwechsel annehmen, der sich in einer ständigen Wasseraufgabe (bei der Excretion) und einer entsprechenden Wasseraufnahme äussert. Diese Wasseraufnahme kann durch den Darm oder durch die Haut erfolgen. Sind äusseres und inneres Medium nicht isotonisch, wie z.B. bei den Süßwassertieren und den marinen Knochenfischen, so kann außerdem ein passiver Ausgleich auf osmotischem Wege durch die Haut eintreten. Bei den wasserlebenden Organismen besteht also im Falle einer Verschiedenheit der beiden genannten Medien die dauernde Gefahr einer Änderung der Zusammensetzung des inneren Mediums. Eine Erhöhung oder Herabsetzung der Molarkonzentration der Körpersäfte kann aber schwere Schädigungen zur Folge haben. Form und Volumen einer Zelle (mit semipermeablen Membranen) sind ja nur so lange konstant, als sie sich in einem isotonischen Medium befindet; ändert sich die Molarkonzentration desselben, so treten Schrumpfungen oder Quellungen auf. Dementsprechend sind bei zahlreichen Wassertieren komplizierte Mechanismen gefunden worden, die dazu dienen, die Molarkonzentration der Körpersäfte auf einer konstanten Höhe unabhängig von der des Aussenmediums zu erhalten.

Die Auffassungen über die Art dieser osmoregulatorischen Einrichtungen haben sich im Laufe der letzten Jahre grundlegend geändert. Insbesondere haben wir wichtige neue Einblicke in die Funktion der an das Aussenmedium angrenzenden Oberflächen und ihre Permeabilität gewonnen. Eine Fülle neuer Fragen ist aufgetaucht, die allerdings noch zum Teil ihrer Bearbeitung harren. Es soll deshalb im Folgenden versucht werden, eine kurzgefasste Darstellung unserer jetzigen Auffassung von der Natur der osmoregulatorischen Mechanismen der Wassertiere zu geben und dabei insbesondere der Unterschied zwischen der bisher herrschenden und der augenblicklichen Meinung herausgearbeitet werden. Um den Umfang der Arbeit nicht zu gross werden zu lassen, sollen nur die *wasseratmenden* Tiere (mit Einschluss der Amphibien), nicht aber die im Wasser lebenden und *luftatmenden* Organismen, wie die Wasserkäfer und die wasserlebenden Säuger, berücksichtigt werden.

II. DIE MOLARKONZENTRATION DER KÖRPERSÄFTE BEI NORMALEM AUSSENMEDIUM.

Als Mass für die Molarkonzentration, bzw. "den osmotischen Druck" einer Lösung kann man die Gefrierpunktserniedrigung derselben gebrauchen¹. Die beifolgende Tab. I gibt die Ergebnisse derartiger Untersuchungen wieder; sie ermöglicht einen Vergleich zwischen den Konzentrationen im Blut der untersuchten Wassertiere und ihrem normalen Aussenmedium. Bottazzi, Frédéricq u.a. haben aus den angegebenen Werten den Schluss gezogen, dass die Körperflüssigkeiten der marinen Evertebraten isotonisch mit dem Meerwasser seien, in welchem diese Tiere gerade leben, einerlei welches die molekulare Konzentration desselben sei. Die zahlreichen in der Literatur verstreuten Angaben ermöglichen nur schwer eine sichere Entscheidung über die Richtigkeit dieser Ansicht, die in fast sämtlichen modernen Lehrbüchern und Zusammenfassungen wiedergegeben wird (v. Buddenbrock, Hoeber, Jordan, Parnas, Tschermak). Namentlich die Bestimmungen von Bottazzi, dem Entdecker dieses "Gesetzes" selbst, sind jedoch in dieser Hinsicht wenig brauchbar, da dieser Autor fast nie gleichzeitig Innen- und Aussenmedium untersucht hat, sondern neben den Werten für die Körperflüssigkeiten seiner aus dem Golf von Neapel stammenden Versuchstiere meist nur ganz allgemein die in dem Wasser des betreffenden Meeresabschnittes vorkommenden minimalen und maximalen Gefrierpunktserniedrigungen bzw. einen Mittelwert angibt. Aus einigen wenigen seiner Bestimmungen und besonders aus denen von Monti (s. Tab. I) lässt sich jedoch entnehmen, dass die Körperflüssigkeiten der marinen Evertebraten zumindest gelegentlich etwas hypertonisch gegenüber dem Seewasser sind, in dem sie leben. Für die Echinodermen gibt Bottazzi selbst an einer Stelle (Wintersteins *Handbuch der vergleichenden Physiologie*, Bd. I, I. Hälfte, S. 518) an, dass in einigen Fällen der osmotische Druck ihrer Körperflüssigkeiten "ein klein wenig grösser" sei als der des umgebenden Meerwassers. Die Differenz ist aber anscheinend meist so klein, dass sie nur bei sehr exakten Bestimmungen

¹ Die Gefrierpunktserniedrigung dividiert durch 185 (die molekulare Gefrierkonstante des Wassers) ergibt den Molengehalt.

Die Osmoregulation wasserlebender Tiere

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Tabelle I. Gefrierpunktserniedrigungen der Körpersäfte. (°C.)

Species	Innenmedium	Aussenmedium	Autor
Meeresbewohner			
Coelenterata			
<i>Alcyonium palmatum</i>	2.195-2.196	2.2	Bottazzi
Echinodermata			
<i>Asterias glacialis</i>	2.295	2.195-2.36	"
<i>Holothuria poli</i>	2.299	2.195-2.36	"
Annelida			
<i>Sipunculus nudus</i>	2.27-2.31	2.29	"
<i>Aphrodite aculeata</i>	2.259	2.29	"
Mollusca			
<i>Aplysia limacina</i>	2.32	2.195-2.360	
<i>Cassis sulcosa</i>	2.36	2.22	Monti
<i>Ostrea edulis</i>	2.23	2.11-2.14	"
<i>Mytilius edulis</i>	2.26	2.11-2.14	"
<i>Octopus vulgaris</i>	2.16	2.11-2.14	"
Arthropoda			
<i>Limulus polyphemus</i>	1.90	1.82	n. Rogers
<i>Homarus vulgaris</i>	2.29	2.269-2.278	Bottazzi
<i>Homarus americanus</i>	1.82	1.80	n. Rogers
<i>Hyas aranea</i>	1.83	1.80	Schlieper
<i>Carcinus maenas</i>	2.17	1.96-1.99	Monti
<i>Maja verrucosa</i>	2.13	2.17	Frédéricq
Tunicata			
<i>Ascidia mentula</i>	2.08	1.98	Duval et Prenant
Elasmobranchiata			
<i>Scyllium canicula</i>	2.22	2.15	Duval
<i>Mustellus vulgaris</i>	2.36	2.29	Bottazzi
<i>Carcharias littoralis</i>	2.03	1.83	n. Rogers
<i>Trygon violacea</i>	2.43	2.29	Bottazzi
<i>Raja undulata</i>	1.89	1.84	Duval
<i>"</i>	1.96	1.88	"
Teleostei			
<i>Conger vulgaris</i>	0.77	2.14	Dakin
<i>Pleuronectes platessa</i>	0.787	1.9	Bottazzi
<i>Charax punctaco</i>	1.04	2.29	"
<i>Cerna gigas</i>	1.034	2.29	
Süßwasserbewohner			
Mollusca			
<i>Anodonta cygnea</i>	0.09	—	W. Koch
<i>Unio pictorum</i>	0.15	—	n. Monti
<i>Limnaea stagnalis</i> (Gewebsshaft)	0.22-0.23	0.02-0.03	Frédéricq
Annelida			
<i>Hirudo officinalis</i> (Gewebsshaft)	0.43	—	"
Crustacea			
<i>Daphnia magna</i>	0.20-0.67	—	Fritzsche
<i>Potamobius astacus</i>	0.80	—	Frédéricq
<i>Telphusa fluviatile</i>	1.17	—	Duval
<i>Eriocheir sinensis</i>	1.09	—	Schlieper
Teleostei			
<i>Barbus fluviatilis</i>	0.50	—	Frédéricq
<i>Leuciscus gobius</i>	0.45	—	"
<i>Cyprinus carpio</i>	0.50	—	Duval
<i>Salmo fario</i>	0.57	—	Dekhuyzen
<i>Anguilla anguilla</i>	0.62	—	Duval

zutage tritt und deshalb wohl von fast sämtlichen Autoren, die sich mit diesem Stoff beschäftigt haben, übersehen bzw. nicht gewertet worden ist. Bei einem dekapoden Krebs, *Carcinus maenas*, ist eine geringe Hypertonie des Innenmediums stets festzustellen (s. Tab. II).

Tabelle II. *Carcinus maenas* (nach Schlieper, 1929 c).

Blut Δ (°C.)	Aussenmedium Δ (Nordseewasser) (°C.)
1.97	1.92
1.95	1.92
2.00	1.91
1.96	1.90
1.94	1.90

Auch für die Ascidiens ist das Gleiche von Duval et Prenant (1926) gezeigt worden. Für die Elasmobrachier wurde bisher von Bottazzi u.a., ebenso wie bei den marinen Evertebraten, Isotonie zwischen Innen- und Aussenmedium behauptet. Insbesondere die sorgfältigen Untersuchungen von M. Duval (1925) haben jedoch auch in diesem Fall eine geringe aber deutliche Hypertonie der Körpersäfte gegenüber dem umgebenden Meerwasser festgestellt (s.a. E. Rodier, 1899).

Im Gegensatz zu den bisher erwähnten Tiergruppen besteht bei den marinen Knochenfischen und bei sämtlichen Süsswassertieren eine starke Anisotonie der Körpersäfte gegenüber dem äusseren Medium. Bei den im Meere lebenden Teleostiern beträgt die Molarkonzentration des Blutes etwas weniger als die Hälfte von der des Seewassers. Das innere Medium ist also in diesem Falle hypotonisch gegenüber dem Aussenmedium. Anders verhalten sich die Süsswassertiere, bei ihnen sind die Körpersäfte stets stark hypertonisch gegenüber dem Aussenmedium. Im Einzelnen variieren die Werte aber sehr; die niedrigsten Konzentrationen scheinen bei den Süsswassermollusken und Protozoen (*ca.* 0.08 Mol) vorzukommen.

Es existiert demnach anscheinend bei sämtlichen wasserlebenden Tieren ein Bestreben, die Molarkonzentration der Körpersäfte auf einer gewissen Höhe über oder unter der des Aussenmediums zu halten. Bei den marinen Wirbellosen scheint diese Tendenz nur sehr schwach entwickelt zu sein, während sie bei den anderen Tiergruppen deutlicher in Erscheinung tritt. Bei den Süsswassertieren ist die Hypertonie der Körpersäfte gegenüber dem Aussenmedium bei der geringen Elektrolytkonzentration desselben ja direkt eine Lebensnotwendigkeit. Die hohe im Meerwasser vorhandene Salzkonzentration scheint andererseits auch nicht optimal zu sein, da, wie gesagt, sämtliche marinen Teleostier eine niedrigere Konzentration in ihrem Blute aufrecht erhalten. Von den regulatorischen Mechanismen, die bei den besprochenen Tiergruppen die osmotische Unabhängigkeit des inneren Mediums gegenüber dem Aussenmedium aufrecht erhalten, wird erst weiter unten die Rede sein. Hinweisen möchte ich hier nur darauf, dass die Molarkonzentration in den Körpersäften vorwiegend durch einen entsprechenden Elektrolytgehalt (vor

allem NaCl) bedingt wird. Eine Ausnahme von dieser Regel bilden allein die Elasmobranchier; bei ihnen beträgt die Elektrolytkonzentration der Körpersäfte nur 38–48 Prozent von der des umgebenden Meerwassers. Die hohe Molarconzentration des Blutes kommt hier erst durch einen ganz aussergewöhnlich grossen Harnstoffgehalt (2–3 Prozent) zustande. Da aber die Aussenmembranen der Haifische, insbesondere die der Kiemen, für Harnstoff undurchlässig sind (Duval), werden die regulatorischen Mechanismen und die Arbeitsleistung, die notwendig sind, um die geschilderte geringe Hypertonie der Körpersäfte gegenüber dem Aussenmedium aufrecht zu erhalten, vielleicht die gleichen sein wie bei den übrigen Meeresbewohnern mit Ausnahme der Knochenfische.

Anhangsweise möchte ich hier noch die Bewohner der im Binnenland gelegenen hochkonzentrierten Salzwässer, vor allem der sog. Salinen, erwähnen. Medwedewa (1927) hat den weit verbreiteten Salzwasserkrebs *Artemia salina* untersucht und gefunden, dass hier der osmotische Druck der Haemolymphe, ähnlich wie bei den marinischen Knochenfischen, stets geringer ist als der des salzreichen Aussenmediums (s.a. Thompson, 1917, u. Martin and Wilbur, 1921). Bereits bei einem Kochsalzgehalt von nur 2 Prozent im Aussenmedium ist eine Hypotonie des Innenmediums vorhanden (s. Tab. III).

Tabelle III. *Artemia salina* (nach Medwedewa, 1927).

Die Konzentrationen sind ausgedrückt durch die Prozentzahlen
isotonischer Kochsalzlösungen

Aussenmedium	Innenmedium
4·5 8	1·2 1·3

Ebenso aus dem Rahmen des bisher Bekannten fallend, ist das Verhalten von *Pachygrapsus crassipes*, eines im Meer vorkommenden brachyuren Dekapoden. Nach den Untersuchungen von Baumberger and Olmstedt (1928) weisen diese Krabben ähnlich wie die marinischen Knochenfische und auch *Artemia* normalerweise eine Hypotonie ihrer Körpersäfte gegenüber dem Aussenmedium auf (Blut $\Delta = 1\cdot327^\circ\text{C}$., Seewasser des Fundorts $\Delta = 1\cdot975^\circ\text{C}$.). Eigenartigerweise bleibt diese niedrige Molarconzentration des Blutes nicht während des ganzen Lebens konstant, sondern steigt während der Häutung jedesmal beträchtlich und übertrifft dabei sogar für kurze Zeit die des Aussenmediums.

III. DIE MOLARKONZENTRATION DER SÄFTE BEI EIERN UND EMBRYONEN.

Die Säfte der Eier und Embryonen einiger Süßwassertiere haben während bestimmter Entwicklungsstadien nicht den gleichen osmotischen Druck wie die Körpersäfte der erwachsenen Individuen. Backman zusammen mit Runnström und Sundberg (1912), sowie Bialaszewicz (1912), konnten diese eigenartige Erscheinung beim Frosch aufdecken. Der Gefrierpunkt der fertig ausgebildeten Ovarialeier stimmt danach mit dem des Blutes des mütterlichen Tieres einigermassen

überein. Kurze Zeit nach der Befruchtung beträgt der osmotische Druck der Eier jedoch nur einen Bruchteil des anfänglich vorhandenen. Durch die Befruchtung kommt demnach im Ei eine bedeutende Reduktion des osmotischen Druckes zustande, die einige Zeit anhält. Erst am fünften Entwicklungstage tritt eine zuerst schnelle, später langsamere Steigerung des osmotischen Druckes im Ei ein, bis er schliesslich am 30.-35. Tage den für das erwachsene Tier charakteristischen Wert wieder erreicht hat (s. Abb. 1). Untersuchungen von Backman (1912) an *Bufo vulgaris* und *Triton cristatus* hatten die gleichen Ergebnisse. Allgemeine

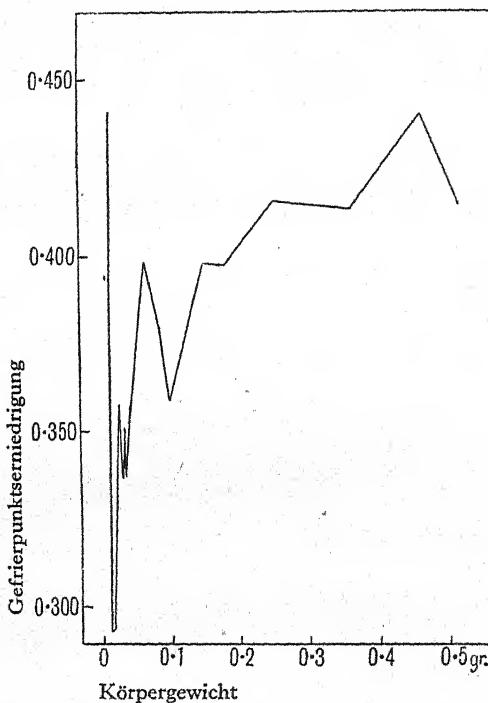


Abb. 1. Veränderung des osmotischen Druckes während der larvalen Entwicklung der Frösche (nach Bialaszewicz).

Verbreitung scheint dieser Erscheinung aber bei den Süßwassertieren nicht zuzukommen. Während der Entwicklung der Salmonideneier ändert sich der osmotische Druck, wie Gray (1916) an der Forelle und Runnström (1920) am Saibling zeigen konnten, nur sehr wenig.

IV. DIE MOLARKONZENTRATION DER KÖRPERSÄFE BEI KÜNSTLICH VERÄNDERTER SALZKONZENTRATION DES AUSSENMEDIUMS.

Einleitung.

Bei allen Versuchen über den Einfluss der Konzentration des Aussenmediums auf die des Innenmediums der Wassertiere ist es fast stets notwendig mit physiologisch aequilibrierten Salzlösungen (Meerwasser oder Ringerlösung) zu arbeiten. Lösungen eines einzigen Salzes (z.B. NaCl) wirken, auch wenn sie in isotonischer

Konzentration verwandt werden, bei vielen Tieren giftig, wie dies Loeb (1903, 1915), Ostwald (1905), Siedlecky (1903), Gueylard (1924) u.a. gezeigt haben. Versetzt man z.B. den kleinen Brackwasserteleostier *Fundulus heteroclitus* in eine reine Na- oder K-Salzlösung, so erkrankt er und stirbt, sobald die Salzkonzentration im Aussenmedium ein gewisses, ziemlich niedriges, für jedes Salz charakteristisches Mass übersteigt; in isotonischem Meerwasser bleibt er dagegen beliebig lange am Leben (Loeb, 1915). F. Krüger (1928) und A. Bethe (1929) führen diese Giftigkeit reiner Salzlösungen für Wassertiere auf ein Eindringen der betreffenden Ionen durch die Körperoberfläche hindurch zurück.

(1) Marine Evertebraten.

Zahlreiche Autoren (Frédéricq, Quinton, Garrey, Henri et Lalou, Duval, u.a.) haben den Einfluss einer künstlichen Veränderung in der Salzkonzentration des Aussenmediums auf den osmotischen Druck der Körpersäfte mariner Evertebraten untersucht. Es sollen danach diese Organismen in osmotischer Hinsicht "ein Spielball ihrer Umgebungsbedingungen" sein (R. Höber, 1926). Bringt man z.B. einen Seestern (*Asterias*) in verdünntes Seewasser, so sinkt die Molarkonzentration seiner Körpersäfte rapid, bis nach wenigen Stunden wieder annähernde Isotonie zwischen Innen- und Aussenmedium besteht. Das Gleiche ist der Fall nach Ueberführung in hypertonisches Seewasser; auch hier ist innerhalb kurzer Zeit eine passive (?) Angleichung an die erhöhte Salzkonzentration des Aussenmediums zu beobachten (s. Tab. IV).

Tabelle IV (nach Frédéricq bezw. Schlieper).

Art	Aussenmedium Δ	Innenmedium Δ	Aufenthaltsdauer
<i>Maja verrucosa</i>	(°C.)	(°C.)	— Stunden
	2·13 normal 1·38 verdünnt	2·17 1·40	
<i>Hyas aranea</i>	2·98 konzentriert	2·94	24 6·3 — Stunden
	1·80 normal 1·19 verdünnt	1·83 1·21	

Aehnliche Feststellungen wurden bei Echinodermen, Gastropoden, Cephalopoden, etc., gemacht. Auch Untersuchungen mit Hilfe einer anderen Methode als der Gefrierpunktsbestimmung, wie z.B. der Chlortitrierung, ergaben die gleichen Resultate. Dass diese Ergebnisse aber nicht etwa Kunstprodukte infolge einer Schädigung bei der plötzlichen Ueberführung in das anisotonische Versuchsmittel sind, zeigte die vergleichende Untersuchung der Körpersäfte mariner Wirbelloser aus Meeresabschnitten mit verschiedenen salzhaltigem Seewasser (s. Tab. V).

Die Ergebnisse derartiger Messungen sind bisher verallgemeinert und auf die gesamten marinern Evertebraten übertragen worden. Bottazzi spricht sogar von einem "Gesetz der Poikilosmotizität der wirbellosen Seetiere." Neuere Untersuchungen zeigten jedoch, dass es von dieser Regel auch Ausnahmen gibt. So bleibt bei gewissen marinern Krebsen nach Ueberführung in verdünntes Seewasser

Tabelle V.

Art	Innen-medium Δ	Aussen-medium Δ (°C.)	Fundort	Autor
<i>Limulus polyphemus</i>	2.025-2.04 1.71	2.03 1.707	Beaufort	Garrey
<i>Mytilus edulis</i>	2.26 0.95	2.11-2.14 0.94	Newport River Mittelmeer	Monti
<i>Arenicola marina</i>	1.70 0.75	1.72 0.77	Ostsee Helgoland	Schlieper
"			Ostsee	"

stets eine deutliche Differenz zwischen Innen- und Aussenmedium bestehen, eine Hypertonie des Blutes, wie sie in dieser Höhe für gewöhnlich nicht existiert (s. Tab. VI).

Tabelle VI.

Art	Aussen-medium Δ (°C.)	Innen-medium Δ (°C.)	Aufenthalts-dauer	Autor
<i>Platycarcinus pagurus</i>	1.10	1.27	42 Stunden	Duval
<i>Cancer pagurus</i>	0.82	1.02	40 "	Schlieper

Noch weit ausgesprochener zeigt dieses Verhalten *Carcinus maenas*, eine Krabbe, die grosse Schwankungen im Salzgehalt des Meerwassers vertragen kann. Bringt man einen solchen Krebs in verdünntes Seewasser, so sinkt wohl die Molarkonzentration seiner Körpersäfte etwas herab, bleibt dann aber auf einer bestimmten Höhe über der des Aussenmediums stehen. Diese Tatsache geht bereits aus einem älteren Versuch Frédéricqs (1904) hervor. Frédéricq fand dabei, dass das Blut eines *Carcinus* nach dreitägigem Aufenthalt in verdünntem Seewasser ($\Delta = 1.19^\circ$) erst bei -1.68° C. gefror. Bei der relativ kurzen Versuchsdauer blieb es allerdings unklar, ob hier ein aktives Festhalten der inneren Molarkonzentration vorlag, oder ob es sich nur um einen besonders langsamen Ausgleich—etwa infolge einer geringeren Durchlässigkeit der Haut—handelte. Neuere Versuche von M. Duval (1926) sprechen für die erstere Möglichkeit. Duval hielt die Krabben 15 Tage lang in verdünntem Seewasser und fand, dass der Unterschied in der Molarkonzentration des Innen- und Aussenmediums ebenso gross war, wie nach einem nur 24-stündigem Aufenthalt in dem gleichen Seewasser (s. Tab. VII).

Tabelle VII. *Carcinus maenas*.

Aussen-medium Δ (°C.)	Innen-medium Δ (°C.)	Aufenthalts-dauer	Autor
1.39	1.75	26 Stunden	Duval
1.39	1.75	15 Tage	"
0.63	1.30	26 Stunden	"
0.63	1.28	15 Tage	"

Bringt man dagegen *Carcinus* in hypertonisches Seewasser, so steigt die Molarkonzentration des Blutes entsprechend der des äusseren Mediums (Frédéricq,

Duval). Ohne die Untersuchungen von Duval zu kennen, kam Schlieper (1929 a) zu den gleichen Ergebnissen (s. Abb. 2). Schlieper untersuchte u.a. die in der Kieler Förde in einem Seewasser von nur 14-17 v.T. Salzgehalt vorkommenden und sich

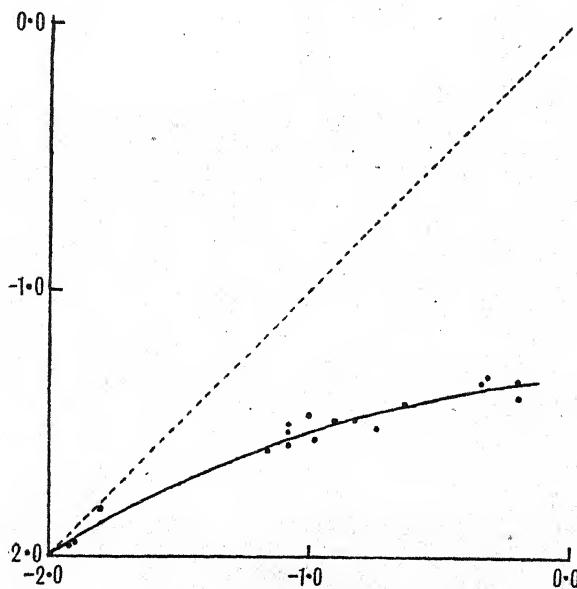


Abb. 2. Gefrierpunktserniedrigungen des Blutes bei *Carcinus maenas* nach Aufenthalt in verschiedenen Salzkonzentrationen. Die gestrichelte Linie gibt den Verlauf der Kurve bei Isotonie zwischen Innen und Außenmedium an. Abszisse: Außenmedium, Ordinate: Innenmedium (nach Schlieper).

auch dort fortpflanzenden Taschenkrebsen. Auch hier lag bei sämtlichen Tieren der Gefrierpunkt der Körpersäfte weit unter dem des Außenmediums (s. Tab. VIII).

Tabelle VIII. *Carcinus maenas* (nach Schlieper).

Wasser des Fundorts Δ (°C.)	Innenmedium Δ (°C.)
0.90	1.45-1.53
0.75	1.48-1.55

Hierdurch ist auf das Klarste bewiesen, dass *Carcinus maenas* in verdünntem Seewasser die Hypertonie seines Blutes gegenüber dem Außenmedium aktiv aufrecht erhält. Ähnliche Eigenschaften kommen auch anderen euryhalinen marinen Evertebraten zu. Bei *Nereis diversicolor* konnte Schlieper (1929 a) feststellen, dass die Körpersäfte erst bei -0.70° C. gefroren, während das Wasser des Fundortes bereits bei -0.21° C. erstarrte. Wurden jedoch die Würmer in höher konzentriertes Meerwasser gebracht, so stieg die Molarkonzentration ihrer Körpersäfte stark an (s. Tab. IX).

Tabelle IX. *Nereis diversicolor* (nach Schlieper, 1929 a).

Aussen-medium Δ (°C.)	Innen-medium Δ (°C.)	Aufenthalts-dauer
0·04	0·50	4 Tage
0·21	0·70	—
0·45	0·86	2 Tage
0·88	1·10	3 "
0·95	1·14	3·5 "

Man kann also wohl mit Recht sagen, dass das von Bottazzi aufgestellte Gesetz der Poikilosmotizität der Meeresvertebraten keine allgemeine Gültigkeit besitzt. Die euryhalinen Meeresswirbellosen, d.h. diejenigen, welche grössere Schwankungen im Salzgehalt des Meerwassers ohne Schaden vertragen können, besitzen in verdünntem Seewasser einen über dem des Aussenmediums stehenden osmotischen Eigendruck ihrer Körpersäfte. Experimentell nachgewiesen ist zwar etwas Derartiges bisher nur für *Carcinus* und *Nereis*, wahrscheinlich gilt aber das Gleiche für die gesamten Brackwassertiere (wie z.B. *Palaemonetes varians*, *Gammarus locusta*, *Balanus improvisus*, *Mya arenaria*, etc.). Für die stenohalinen Evertebraten des Meeres, d.h. diejenigen, welche nur geringe Schwankungen im Salzgehalt des Aussenmediums ertragen können (wie z.B. Echinodermen, Cephalopoden, etc.), scheint dagegen das Gesetz der Poikilosmotizität annähernde Gültigkeit zu besitzen.

(2) Süsswasservertebraten.

Nur wenige Untersuchungen existieren über den Einfluss von Salzwasser auf die Molarkonzentration der Körpersäfte süsswasserlebender Wirbelloser (Calugareanu, 1914; Koch, 1917; Duval, 1925). Plötzliche Ueberführung in reines Meerwasser ruft fast stets den Tod dieser Tiere hervor. Einzelne Arten sind jedoch zumindest für einige Zeit in Salzwasser lebensfähig. Besonders dann, wenn die Ueberführung allmählich geschieht, lassen sich verschiedene Muscheln, Schnecken und Krebse an reines Meerwasser gewöhnen (Beudant, 1816; Duval, 1925). Untersucht man die Körperflüssigkeiten derartiger Tiere, so zeigt sich ausnahmslos eine starke Erhöhung der inneren Molarkonzentration (s. Tab. X u. Abb. 3).

Tabelle X. *Anodonta spec.* (nach Duval, 1925).

Aussenmedium Δ (°C.)	Innenmedium Δ (°C.)
0·02	0·10
0·08	0·13
0·24	0·25
0·40	0·40

Aehnliches wie bei *Anodonta* lässt sich bei *Telphusa fluviatile*, einer in Italien im Süsswasser vorkommenden Krabbe beobachten. Nur ist hier die Zeitspanne bis zur Ausbildung eines Gleichgewichtszustandes zwischen dem Innen- und dem veränderten Aussenmedium viel grösser als bei einem marinem Krebs. Während bei

Carcinus das Gleichgewicht bereits nach 24 Stunden vorhanden ist, dauert dies bei *Telphusa* mehrere Tage (Duval, 1925).

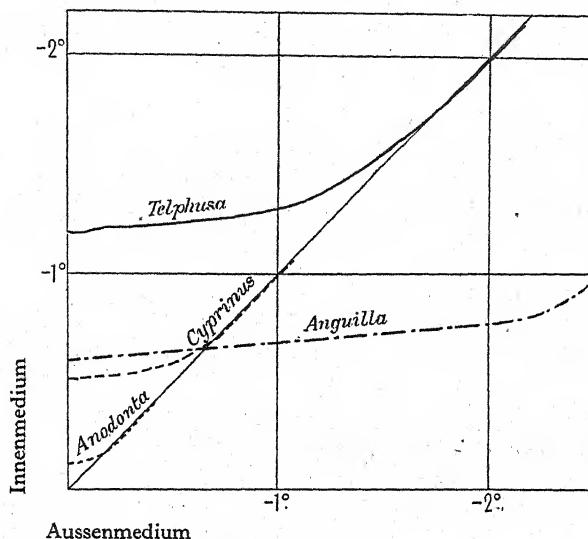


Abb. 3. Molarkonzentration des Blutes verschiedener Süßwassertiere bei Erhöhung des Salzgehaltes im Aussenmedium (nach Duval).

(3) Selachier.

Die Konzentration des Blutes der Knorpelfische hängt weitgehend vom Salzgehalt des Aussenmediums ab. Von den verschiedenen Autoren (Frédéricq, 1904; Garrey, 1905; Portier et Duval, 1922; Duval, 1925), die übereinstimmend diese Tatsache nachweisen, möchte ich nur den letzten, Duval, zitieren. Dieser hielt Haifische in verschiedenen Salzkonzentrationen und auch in reinem Süßwasser. Ich gebe von seinen Ergebnissen nur diejenigen Werte wieder, bei denen ausdrücklich bemerkt ist, dass die untersuchten Exemplare noch keinerlei sichtbare Schädigungen infolge der veränderten Salzkonzentration ihres Aussenmediums erlitten hatten (s. Tab. XI).

Tabelle XI. *Scyllium* (nach Duval, 1926).

Aussenmedium Δ (°C.)	Innenmedium Δ (°C.)	Aufenthaltsdauer
2.08	2.20	6 Stunden 20 Min.
1.47	1.87	6 „ 20 „
1.07	1.76	3 „ 30 „

Bei noch höherem oder niedrigerem Salzgehalt starben die Fische innerhalb ganz kurzer Zeit. Die Dauer der Versuche ist allerdings zu kurz, um annehmen zu können, dass bereits ein Gleichgewichtszustand zwischen Innen- und Aussenmedium vorhanden war. Bei langfristigeren Versuchen würde man—vorausgesetzt,

dass die Fische am Leben geblieben wären—wohl noch grössere Verschiebungen der Molarkonzentration des Blutes beobachtet haben. Die im Laboratorium gewonnenen Ergebnisse werden durch Beobachtungen von Dakin (1912) an frei lebenden Selachien bestätigt. Er konnte auf einer Reise von Kiel durch das Skagerrak in die Nordsee an frischgefangenen Exemplaren folgende Werte feststellen:

Spezies	Aussenmedium Δ (°C.)	Innenmedium Δ (°C.)
<i>Raja radiata</i>	1.66	1.51
<i>Raja valomia</i>	1.95	2.0

(4) Stenohaline Süsswasserteleostier.

Nur wenige der im Süsswasser vorkommenden Knochenfische sind euryhalin, d.h. lebensfähig in salzhaltigem Wasser. Die meisten sterben kurze Zeit (wenige Stunden) nach Ueberführung in Seewasser. Deshalb sind auch die Untersuchungen über den Einfluss einer erhöhten Salzkonzentration des Aussenmediums auf die Molarkonzentration des Blutes dieser Organismen so selten. Es hat selbstverständlich keinen grossen Wert, bei einem solchen sterbenden, nur wenige Stunden mit dem Versuchsmedium in Berührung gewesenen Fisch die Molarkonzentration des Blutes zu untersuchen. Die Wahrscheinlichkeit, dass sich in dieser kurzen Zeit ein Gleichgewicht zwischen Innen- und Aussenmedium herausgebildet hat, ist sehr gering. Duval (1925) hat derartige Untersuchungen am Karpfen (*Cyprinus carpio*) gemacht und gefunden, dass die Konzentration des Innenmediums in keiner Weise eine konstante Grösse ist, sondern von der des Aussenmediums abhängt. Isotonisches und schwach hypertonisches Seewasser ruft hier anscheinend noch keinerlei Schädigungen hervor, während noch höhere Salzkonzentrationen dagegen sehr schnell den Tod herbeiführen. Mit zunehmendem Salzgehalt im Aussenmedium steigt auch die Molarkonzentration des Innenmediums; bereits in isotonischem Seewasser ist die Blutkonzentration erhöht (s. Tab. XII u. Abb. 3).

Tabelle XII. *Cyprinus carpio* (nach Duval, 1925).

Aussen-medium Δ (°C.)	Innen-medium Δ (°C.)	Aufenthaltsdauer	Zustand des Fisches bei Schluss des Versuches
0.02	0.50	—	Sehr gut
0.51	0.59	3.5 Stunden	"
0.68	0.64	4.5 "	"
0.88	0.71	5.5 "	Schlecht
1.02	0.82	1.25 "	"

Duval gelang es aber auch, Karpfen durch ganz allmählichen Zusatz von Seesalz zum Versuchsmedium im Laufe mehrerer Wochen an Salzwasser zu gewöhnen. Bei diesen Fischen, die 12–15 Tage mit dem langsam mit Salz angereicherten Medium in Berührung gewesen waren, war von einem bestimmten Salzgehalt im

Aussenmedium ab Isotonie zwischen Innen- und Aussenmedium vorhanden (s. Tab. XIII).

Tabelle XIII. *Cyprinus carpio* (nach Duval, 1925).

Aussenmedium Δ (°C.)	Innenmedium Δ (°C.)	Versuchsdauer
0.02	0.50	—
0.48	0.57	21 Tage
0.62	0.65	13 "
0.84	0.83	48 "
1.04	1.06	12 "

Dieses Ergebnis beweist, dass sich während der kurzen Zeittdauer der zuerst geschilderten Versuche—bei plötzlicher Ueberführung in Salzwasser—noch kein Gleichgewicht zwischen Innen- und Aussenmedium herausgebildet hatte; es zeigt fernerhin, dass der Karpfen wohl im Süßwasser einen osmotischen Eigendruck in seinem Inneren aufrecht erhalten kann, nicht aber dazu in hypertonischen Salzlösungen imstande ist.

(5) Stenohaline Meeresteleostier.

Die grosse Menge der marinen Knochenfische ist stenohalin, d.h. verträgt nur geringe Schwankungen im Salzgehalt des Aussenmediums. Nach Ueberführung in Süßwasser sterben sie im allgemeinen im Laufe weniger Stunden (P. Bert). Duval (1925) hat verschiedene Mittelmeerteleostier (*Scorpaena*, *Conger*, *Murena*) in Brack- bzw. Süßwasser überführt und nach 5–25 Stunden untersucht. Es zeigten sich dabei keine oder nur sehr geringe Änderungen der Molarkonzentration des inneren Mediums. Da es wegen der kurzen Versuchsdauer nicht sicher ist, ob bereits ein Gleichgewichtszustand zwischen Innen- und Aussenmedium vorhanden war, möchte ich aus diesen Ergebnissen keinerlei Schlüsse ziehen.

(6) Euryhaline Teleostier.

Sowohl im Meer wie auch im Süßwasser gibt es zahlreiche euryhaline Knochenfische, die auch grössere Schwankungen im Salzgehalt ihres Aussenmediums vertragen können. Dazu gehören selbstverständlich sämtliche Wanderfische, die wie der Aal (*Anguilla anguilla*), der Lachs (*Salmo salar*), etc., im Meer und im Süßwasser leben. Von den echten Süßwasserfischen wäre noch besonders der Stichling (*Gasterosteus aculeatus*) zu erwähnen, der auch im Brackwasser lebensfähig ist. Von den marinen Fischen möchte ich nur den von verschiedenen Autoren untersuchten *Fundulus heteroclitus* und die Scholle (*Pleuronectes platessa*) nennen, die beide keineswegs an den hohen Salzgehalt des Meerwassers gebunden sind. Die bisherigen Untersuchungen haben ergeben, dass diese Fische zwar nicht ganz unabhängig von dem Salzgehalt des Aussenmediums sind, dass sie aber doch die Molarkonzentration ihrer Körpersäfte bei weitem fester halten, als die bisher besprochenen Teleostier. So konnte Dakin (1915) zeigen, dass das Blut von *Pleuronectes platessa* je nach dem Fangort (ob Nordsee oder Ostsee) eine etwas verschiedene Molarkonzentration besitzt. Beim Aal fand derselbe Autor, dass das Blut von Süßwasser-

tieren bei -0.57°C . gefriert, während bei Helgoland im Meere gefangene Aale einen weit niedrigeren Erstarrungspunkt aufwiesen (Innenmedium $\Delta = 0.635^{\circ}\text{C}$., Außenmedium $\Delta = 1.9^{\circ}\text{C}$.). Duval (1925) macht, ohne die Untersuchungen von Dakin zu kennen, auf Grund langfristiger Versuche ähnliche Angaben (s.a. Quinton, 1904; Dekhuyzen, 1905; Schmidt-Nielsen, 1909):

$$\left. \begin{array}{l} \text{In Süßwasser gehaltene Aale, Blut } \Delta = 0.62^{\circ}\text{C}. \\ \text{In Seewasser gehaltene Aale, Blut } \Delta = 0.69^{\circ}\text{C}. \\ \qquad \qquad \qquad \text{Meerwasser } \Delta = 2.15^{\circ}\text{C}. \end{array} \right\}$$

(s.a. Abb. 3). Untersucht man jedoch einen aus Süßwasser stammenden Aal kurze Zeit (30 Stunden) nach der Ueberführung in Seewasser ($\Delta = 2.08^{\circ}$), so liegt der Gefrierpunkt des Blutes noch tiefer, bei -0.78° . Demnach tritt unter dem Einfluss des Seewassers zunächst eine relativ starke Erhöhung der Molarkonzentration des Blutes ein, die dann langsam zum Teil wieder verschwindet. Etwas ganz Aehnliches lässt sich bei dem von Loeb and Wasteneys (1916) untersuchten

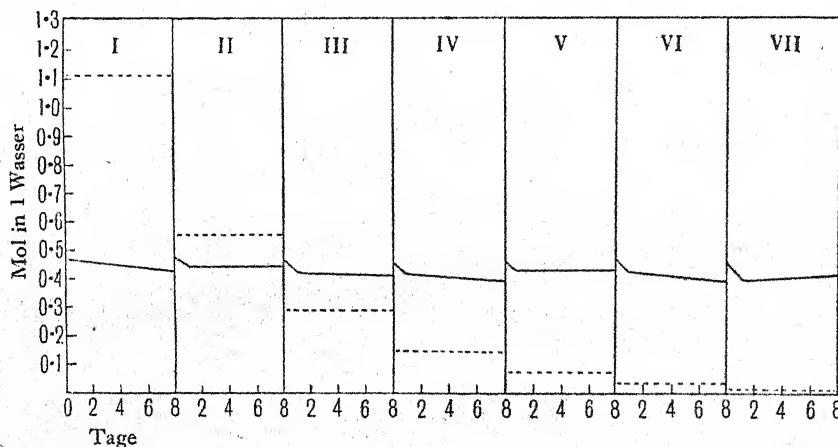


Abb. 4. Molarkonzentration im Blut von *Fundulus heteroclitus* und ihre Beeinflussung durch die Molarkonzentration des Außenmediums in 8-tägigen Versuchen. Die Tiere wurden aus normalem Meerwasser in künstliches verschiedener Konzentration gebracht. — Molarkonzentration des Blutes; - - - Molarkonzentration des Außenmediums (aus Parnas nach Loeb u. Wasteneys).

Fundulus heteroclitus nachweisen. Bringt man diesen Fisch aus normalem verdünntes Seewasser, so sinkt am ersten Tage die Molarkonzentration der Körpersäfte etwas ab, nach 8 Tagen hat sich dieser Unterschied aber annähernd wieder ausgeglichen (s. Abb. 4). Aehnliche Ergebnisse zeigen auch die Versuche von F. Gueylard (1924) am Stichling (*Gasterosteus leirurus*). Im Grossen und Ganzen können wir also sagen, dass die euryhalinen Teleostier einen osmotischen Eindruck ihrer Körpersäfte sowohl im Süßwasser wie im Salzwasser aufrecht erhalten können. Im Einzelnen ist jedoch diese Fähigkeit bei den verschiedenen Arten verschieden entwickelt.

(7) Amphibien.

Zahlreiche Autoren haben den Wasserhaushalt der Amphibien, besonders den des Frosches, studiert. Schon Claude Bernard (1859) zeigte, dass Frösche sehr bald sterben, wenn sie in Salzwasser verbracht werden. Nach P. Bert (1871) büßen sie in Meerwasser von 3·2 v.H. Salzgehalt innerhalb einer Stunde ihr Leben ein. In einer 1 prozentigen NaCl-Lösung dagegen können sie beliebig lange Zeit existieren (Semper, 1880).

Gefrierpunktsbestimmungen ergaben, dass bei einer Erhöhung des Salzgehaltes im Aussenmedium auch die Molarkonzentration der Körpersäfte ansteigt, wobei die Tendenz vorhanden zu sein scheint, die Konzentration des Blutes etwas über der des Aussenmediums zu halten, während die in den Lymphsäcken enthaltene Flüssigkeit dauernd leicht hypotonisch ist (s. Tab. XIV).

Tabelle XIV. *Rana esculenta* (nach Brunacci, 1912).

Aussen-medium Δ	Körperflüssigkeit Δ	Bemerkung
Aqua dest.	0·430°	Blut (nach Bottazzi)
0·650°	0·700°	Blut, defibriniert
0·650°	0·645°	Lymphsackflüssigkeit
0·700°	0·740°	Blut, defibriniert
0·700°	0·695°	Lymphsackflüssigkeit

Die untersuchten Tiere (Sommerfrösche) wurden 3–8 Tage in den verschiedenen Lösungen gehalten. Ein Gleichgewichtszustand zwischen Innen- und Aussenmedium bildete sich relativ rasch, in 10–11 Stunden, heraus. Als Maximalwert fand Brunacci einen Gefrierpunkt von –0·78° C. im Blut bei Einwirkung von hypertoner Ringerlösung ($\Delta = 0\cdot775^\circ$ C.). Für Kröten (*Bufo vulgaris*) und Molche (*Triton cristatus*) liegen Ergebnisse vor, die auf Ähnliches hinweisen (Backman und Sundberg). Demnach sind die Amphibien wohl im Süßwasser imstande, den osmotischen Druck ihrer Körpersäfte auf einer konstanten Höhe zu halten, nicht aber in Salzlösungen.

V. DIE OSMOREGULATORISCHEN MECHANISMEN.

Einleitung.

Die osmoregulatorischen Mechanismen der Wassertiere sind heute erst zum kleinsten Teil erforscht. Einzig und allein die Physiologie des Wasserhaushaltes der Amphibien ist uns auf Grund zahlreicher Arbeiten, vor allem aus den letzten Jahren, einigermassen bekannt. Bei den meisten übrigen Tiergruppen sind wir bis vor kurzem über einige Analogieschlüsse und Arbeitshypothesen noch kaum hinausgekommen. Es kann deshalb nicht Wunder nehmen, wenn sich bei genauer experimenteller Nachprüfung manche der bisherigen Annahmen als unrichtig herausstellen werden. Dies gilt auch—wie ich schon jetzt hervorheben möchte—for die bisherige Auffassung von der Osmoregulation der Süßwasserevertebraten.

(1) *Protozoen.*

Ueber den Gefrierpunkt des Zellsafes bei Protozoen liegen bisher infolge der Kleinheit der Objekte keinerlei direkte Bestimmungen vor. Man nimmt jedoch allgemein an, dass bei den *marinen Protozoen*, die ich hier zunächst besprechen will, zumeist eine annähernde Isotonie zwischen Innen- und Aussenmedium besteht. Bringt man nämlich ein solches Protozoon in verdünntes Seewasser, so lässt sich zunächst ein Anschwellen beobachten, dem nach einiger Zeit eine Volumenänderung in entgegengesetztem Sinne folgt, die meist so lange anhält, bis die normale Gestalt wieder erlangt ist (Enriques, 1902). Zur Erklärung dieser Erscheinung nimmt man an, dass während der ersten Phase (Volumenvermehrung) eine osmotische Wasseraufnahme durch die wie eine semipermeable Membran funktionierende *Pellicula* stattfindet, dass dagegen während der Volumenreduktion ein nicht osmotischer Austritt von Wasser und gelösten Salzen durch die jetzt permeable Aussenmembran stattfindet. Man darf sich vielleicht vorstellen, dass durch den Prozess der Wasseraufnahme die anfänglich vorhandene Semipermeabilität der *Pellicula* zerstört worden ist(?) Ganz das Entsprechende lässt sich nach Ueberführung in hypertönisches Seewasser beobachten: zunächst ein Schrumpfen (osmotische Wasserabgabe) und daran anschliessend eine Wiederherstellung des normalen Volumens (Aufnahme von Wasser + Salz aus dem Aussenmedium). Ob bei dieser Wiederherstellung des normalen Volumens "lediglich ein einfaches physikalisches Geschehen vorliegt, oder ein regulatorisches Eingreifen des Organismus anzunehmen ist, entzieht sich noch unserer Kenntnis" (v. Buddenbrock, 1928, S. 530).

Sicher ist jedoch bei gewissen marinen plankontischen Protozoen, wie z.B. bei *Noctiluca miliaris*, ein osmoregulatorischer Mechanismus vorhanden. *Noctiluca* schwillt in verdünntem Seewasser an und schrumpft nach Ueberführung in ein Seewasser, dessen Salzgehalt auf das Doppelte erhöht worden ist (Harvey, 1917). Sie zeigt also genau die gleichen osmotischen Erscheinungen wie alle andern marinen Protozoen. Und doch handelt es sich hier um etwas anderes. *Noctiluca* schwimmt nämlich normalerweise dicht unter der Meeresoberfläche, ist also spezifisch leichter als das Seewasser. In einer Mischung von Meerwasser mit Süßwasser im Verhältnis 5:5 sinkt sie unter, nicht aber in einer Mischung von 6:4. Da weder Luftblasen noch ein grösserer Fettgehalt (12 Prozent der Trockensubstanz, Pratje 1921) als Ursache des geringen spezifischen Gewichtes in Frage kommen, muss der Zellsaft weniger Salze gelöst enthalten als das umgebende Meerwasser, mit anderen Worten, das Innenmedium muss bei *Noctiluca* dem Aussenmedium gegenüber hypotonisch sein. *Noctiluca* ist aber anscheinend auch imstande, aktiv ihr spezifisches Gewicht und damit die Molarkonzentration im Inneren zu verändern und dadurch im Wasser auf- und abzusteigen! In ruhigen Nächten findet man sie an der Oberfläche des Meeres, während sie an windigen Tagen nur in tieferen Schichten anzutreffen ist. Beim Absinken muss sie demnach Salze von aussen aufnehmen, bezw. Wasser austreten lassen, während beim Aufsteigen die umgekehrten Vorgänge stattfinden. Sehr schön zeigt diese Fähigkeit auch folgender Versuch: Bringt man *Noctiluca* in eine Mischung von Seewasser mit Süßwasser im Verhältnis von 5:5,

so sinkt sie zunächst ab, da ihr spezifisches Gewicht jetzt grösser ist als das des umgebenden Mediums; im Laufe der nächsten Stunde schwilzt sie jedoch langsam an und steigt wieder zur Oberfläche empor. Als Ursache dieses Ansteigens kommt die Bewegung des Tentakels nicht in Frage. Würde *Noctiluca* nur so lange Wasser aufnehmen bis Isotonie zwischen Innen- und Aussenmedium herrscht, so dürfte sie nicht emporsteigen, sie muss also noch über diesen Punkt hinaus Wasser aufnehmen, bezw. Salze hinaustreten lassen. Dieser Versuch bietet also einen klaren Beweis für die Existenz eines osmoregulatorischen Mechanismus, der im allgemeinen bestrebt ist, eine Hypotonie des Innenmediums gegenüber dem Aussenmedium aufrecht zu erhalten, der aber auch den osmotischen Druck des Zellsaftes nach beiden Richtungen verändern kann. Dieser Mechanismus funktioniert nur innerhalb der lebenden Zelle; stirbt eine *Noctiluca*, so sinkt sie unter Wasseraufnahme (Anschwellen) zu Boden. Die geschilderten Beobachtungen, die wir Harvey (1917) verdanken, wurden von Ludwig (1928) bestätigt und erweitert. Harvey hatte bei *Noctiluca* bei Ueberführung in auf die Hälfte verdünntes Seewasser beobachtet, dass das eingedrungene Wasser sich in Vakuolen sammelte, die dann ausgestossen wurden. Er verglich diese Bildungen mit den kontraktilen Vakuolen der Süsswasserinfusorien. Ich möchte diese Annahme aber nur mit Vorbehalt wiedergeben, da Ludwig derartige Vakuolen nur bei absterbenden Exemplaren beobachten konnte. Die Zellmembran von *Noctiluca* ist nach diesem Autor normalerweise, d.h. bei ausgestrecktem Protoplasmanetz, impermeabel für Salze und Farbstoffe. Der Zellsaft reagiert basisch, das Zentralplasma dagegen sauer gegen Nilblausulfat. Dieser verschiedene H-Ionengehalt bedingt nach Ludwig an der Grenzfläche Zellsaft-Zentralplasma ein Adsorptionspotential, das antiosmotischen Wassereinstrom aus dem Zentralplasma in den Zellsaft zur Folge hat.

Bei den Süsswasserinfusorien liegen die Verhältnisse ganz anders. Während bei den marinen Protozoen, mit Ausnahme der zuletzt geschilderten Planktonformen, wie *Noctiluca*, der Zellsaft wahrscheinlich annähernd die gleiche Molarkonzentration wie das umgebende Medium besitzt, ist bei den Süsswasserinfusorien das Innenmedium stets hypertonisch. Zahlreiche Autoren nehmen nun an, dass die bei ihnen allgemein verbreiteten *kontraktilen Vakuolen* osmoregulatorisch funktionieren, d.h. für die Entfernung osmotisch eingedrungenen Wassers sorgen. Eine ganze Anzahl Tatsachen sprechen für die Richtigkeit dieser Annahme. Vor allen Dingen bestehen wichtige Beziehungen zwischen dem Vorkommen kontraktiler Vakuolen und der Konzentration des Aussenmediums. Zahlreiche Meeresprotozoen (die meisten Flagellaten, Rhizopoden, Tintinniden, verschiedene Ciliaten) besitzen keine kontraktilen Vakuolen; ebenso fehlen sie völlig bei den parasitischen Flagellaten und Rhizopoden und bei der rein parasitischen Klasse der Sporozoen (Doflein-Reichenow, 1929). Wo bei den marinen Protozoen kontraktile Vakuolen vorkommen (viele Ciliaten) ist die Schlagfolge meist geringer als bei den Süsswasserinfusorien (s. Tab. XV) (s.a. Doflein-Reichenow, Bd. I, 1929, S. 170). "Die grosse Differenz in der Schlagfolge ist leicht dadurch zu erklären, dass bei marinen Infusorien die Tätigkeit der Vakuole nur der Entfernung der Stoffwechselprodukte dient, während der Organismus in osmotischem Gleichgewicht mit der Umgebungsflüssigkeit

Tabelle XV (aus Parnas).

Marine Infusorien	Frequenz	Süßwasserinfusorien	Frequenz
<i>Lagyrus crassicolis</i>	2 Min.	<i>Chilodon cucullus</i>	5 Sek.
<i>Acinaria incurrenta</i>	6-12 "	<i>Glaucoma colpidium</i>	12-15 "
<i>Cryptochilum echini</i>	20 "	<i>Euplyes charon</i>	31 "

steht, dagegen bei den Süßwasserinfusorien das Protoplasma unter Leistung osmotischer Arbeit dauernd aufgesaugtes Wasser in die Vakuole sezerniert und entleert" (Parnas).

Erhöht man bei einem Süßwasserprotozoon allmählich den Salzgehalt des Aussenmediums, so tritt zunächst keinerlei Veränderung der Körperzellform ein, dann aber—bei etwas höheren Konzentrationen—kann man bald ein Schrumpfen beobachten (bei *Glaucoma colpidium* in 0.075 Mol NaCl). Man kann auf diese Weise eine ungefähre Vorstellung von der Konzentration des Zellsaftes der Süßwasserprotozoen gewinnen; man geht wohl recht in der Annahme, dass bei Eintritt des Schrumpfens die Konzentration des Aussenmediums die des Innenmediums gerade etwas übersteigt. Verschiedene Autoren gewöhnten Süßwasserprotozoen an Salzwasser und konnten dabei, ohne dass irgendwelche Schädigungen sichtbar wurden, stets eine Abnahme der Pulsationsfrequenz der kontraktilen Vakuolen beobachten. Diese Tatsache spricht ebenfalls für eine osmoregulatorische Tätigkeit dieser Organellen bei den Süßwasserinfusorien. M. Zuelzer (1910) erhöhte bei *Amoeba verrucosa* durch langsamem Zusatz von Seewasser im Laufe mehrerer Wochen die Salzkonzentration des Aussenmediums. Bei 0.3-0.8 v.H. Salzgehalt begannen die Pulsationen der kontraktilen Vakuolen langsamer zu werden, und bei 1.5 v.H. Salzgehalt verschwand die kontraktile Vakuole ganz, um erst nach Zurückbringen in Süßwasser innerhalb 24 Stunden wieder zu erscheinen. Einwandfrei erscheinen auch die ähnlichen Untersuchungen A. Herfs' (1922). Dieser Autor hielt verschiedene Protozoen (*Paramaecium*, *Gastrostyla*, *Nyctotherus*, *Balantidium*) in Kochsalz bzw. Ringerlösung (0.25-1.5 Prozent NaCl). Eine Giftwirkung von Seiten dieser Salzwirkung schien nicht zu bestehen, da bei sämtlichen Infusorien die Körperform (durch Schrumpfen oder Quellen) nicht verändert wurde, auch blieb eine normale Beweglichkeit und desgleichen die Teilungsfähigkeit erhalten. Höhere NaCl-Konzentrationen (über 0.75 Prozent) setzten die Teilungsfähigkeit stark herab; niedere Konzentrationen (0.25 u. 0.50 Prozent) schienen den entgegengesetzten Effekt zu haben. Ich gebe in der folgenden Tabelle, die von Herfs für *Paramaecium* gefundenen Werte (Mittelwerte) an.

Tabelle XVI. *Vakuolenfrequenz bei Paramaecium caudatum (nach Herfs)*.

Konzentration (% NaCl)	Zeit zwisch. zwei Entleerungen (Sek.)	In einer Stunde entleert (Körpervolumen)	Temperatur
0.0	6.2	4.8	20-23°
0.25	9.3	2.82	20-22°
0.5	18.4	1.38	19-20°
0.75	24.8	1.08	19-20°
1.0	163.0	0.16	19-20°

Auf Grund dieser und ähnlicher Versuche scheinen verschiedene Autoren der Ansicht zu sein, dass die Tätigkeit der kontraktilen Vakuolen den Protozoen das Leben im Süsswasser überhaupt erst ermögliche; ohne sie müsste das durch die semipermeable Körperhülle eindringende Wasser die Körpermasse zum Aufquellen bringen und die Funktionsweise des Protoplasma stören (R. Hesse, 1924, S. 29–30). Dieser Schluss erscheint mir in verschiedener Beziehung zu weitgehend. Einmal liegt bisher noch kein einziger zwingender Beweis für eine einfache Semipermeabilität der Protoplasmahülle der Protozoen vor. Der ohne Zweifel bei den Süsswasserinfusorien vorhandene Wasserdurchstrom kann auch auf einer aktiven Wasseraufnahme (u. Ausscheidung) von Seiten des Organismus beruhen. Dann fehlen aber auch noch Messungen des osmotischen Druckes der Vakuolenflüssigkeit, die allerdings infolge der Kleinheit der Objekte kaum jemals durchzuführen sein werden. Vielleicht sind Versuche von A. Gruber (1899) geeignet, in dieser Hinsicht einigen Aufschluss zu geben. *Actinophrys sol*, mit welcher Gruber arbeitete, kommt sowohl im Meer als auch im Süsswasser vor. Die Süsswasserindividuen besitzen ein lockeres Plasma von blasiger Struktur und durchweg kontraktile Vakuolen. Die marine Varietät hat im Gegensatz dazu ein dichtes körniges Plasma und keine kontraktile Vakuolen. Gruber konnte nun aus Meerwasser stammende Individuen an Süsswasser gewöhnen und mehrere Wochen in diesem Medium halten. Dabei nahm das Plasma schon nach kurzer Zeit die blasige Beschaffenheit der Süsswasserform an, kontraktile Vakuolen traten jedoch in keinem Falle auf. Es unterliegt also keinem Zweifel, dass Protozoen im Süsswasser zumindest für einige Zeit existieren können, ohne im Besitz kontraktiler Vakuolen zu sein. Etwas ähnliches konnte Herfs (1922) an der im Froschdarm parasitierenden *Opalina ranarum*, die ja auch keine kontraktilen Vakuolen besitzt, beobachten. Er hielt *Opalina* 8–10 Tage in reinem Süsswasser, ohne dass sich grössere Schädigungen zeigten. Pulsierende Vakuolen traten nicht auf. Herfs sagt selbst dazu: "Diese Beobachtung bedeutet nun sicher eine ernste Schwierigkeit für die Auffassung, in der pulsierenden Vakuole ein Schutzorgan gegen Aussüssung zu sehen." Da aber eine Wasserundurchlässigkeit des Ectoplasma bei *Actinophrys* und *Opalina* wohl nicht in Betracht kommt, kann m.E. aus diesen Versuchen nur auf eine aktiv vom Zellkörper ausgeübte osmotische Resistenz bei Aufenthalt in Süsswasser geschlossen werden. Adolph (1926) hat einen weiteren Beitrag zu dieser Frage geliefert. Er versuchte die Wassermenge exakt zu bestimmen, welche die kontraktile Vakuole von *Amoeba proteus* im Laufe einer bestimmten Zeit ausscheidet. Bei den von ihm in Kochsalzlösungen (m/20) gehaltenen Amoeben war, ebenso wie in den Versuchen von Herfs an *Paramecium*, die Vakuolenfrequenz stark vermindert. Genaue Messungen des Durchmessers der kontraktilen Vakuolen kurz vor der Entleerung ergaben jedoch, dass derselbe bei Aufenthalt der Amoeben in Salzwasser bedeutend grösser war als bei den in Süsswasser gehaltenen Exemplaren, dass also der Füllungsgrad der kontraktilen Vakuolen bei Salzwassertieren den bei Süsswassertieren überstieg. Adolph berechnete nun auf Grund dieser Messungen die Arbeitsleistung der kontraktilen Vakuolen und fand keinerlei Beziehung zwischen der Grösse der Salzkonzentration des Aussenmediums und der pro Zeiteinheit aus-

geschiedenen Wassermenge. Diese Ergebnisse sprechen ebenfalls in jeder Weise gegen die oben auf Grund der Versuche von Zuelzer, Herfs, etc., wiedergegebenen Anschauungen über die Funktion der kontraktilen Vakuolen der Süsswasser-infusorien. Sie beweisen vielmehr, dass—zumindest in diesem Einzelfalle (bei *Amoeba proteus*)—die Stärke des Wassereinstromes von der Konzentration des Außenmediums unabhängig ist und machen einen aktiv von Seiten des Organismen geleiteten Wasserdurchstrom wahrscheinlich. Ob allerdings dieses Ergebnis verallgemeinert und auf die anderen Süsswasserprotozoen übertragen werden darf, muss vorläufig dahingestellt bleiben. Ich möchte aber schon an dieser Stelle darauf hinweisen, dass neuere Untersuchungen am Frosch ganz ähnliche Resultate aufweisen.

Anhangsweise möchte ich noch erwähnen, dass von Spek (1921) die Permeabilität des Ectoplasmas eines Süsswasserrhizopoden, *Actinosphaerium eichhorni*, untersucht worden ist. Spek gelang es, diese Protozoen in Gemischen von NaCl, KCl, CaCl₂ und NaHCO₃ zu züchten. Reine Salzlösungen dagegen wirkten giftig, sie erzeugten binnen kurzem eine mehr oder weniger starke Trübung des normalerweise klaren Protoplasmas und führten innerhalb weniger Tage den Tod der Tiere herbei. Höber (1926, S. 496) möchte auf Grund dieser Versuche annehmen, dass *Actinosphaerium* normalerweise und in äquilibrierten Salzlösungen für Salze undurchlässig (semipermeabel) ist, aber pathologisch durchlässig wird, sobald ein einzelnes Salz prävaliert.

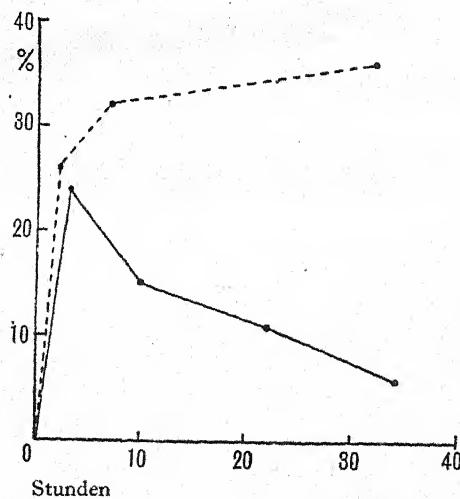


Abb. 5. Gewichtsänderung von *Asterias rubens* und *Nereis diversicolor* nach Ueberführung aus Nordseewasser in verdünntes Seewasser von 20 v.T. Salzgehalt. — = *Nereis*; - - - = *Asterias* (nach Schlieper).

(2) Marine Evertebraten.

Wir haben oben gesehen, dass die *stenohalinen* Evertebraten des Meeres keinen von dem des Außenmediums wesentlich verschiedenen osmotischen Eigendruck aufweisen. Nach Ueberführung in ein anisotonisches Medium ändert sich bei

ihnen die Molarkonzentration der Körpersäfte innerhalb kurzer Zeit derart, dass nach wenigen Stunden ein Ausgleich herbeigeführt ist. Dementsprechend scheinen irgendwelche leistungsfähige osmoregulatorische Einrichtungen bei dieser Tiergruppe nicht zu existieren. Ansätze zu einer aktiven Regulation scheinen nur bei gewissen höheren marinen Wirbellosen, die—wie erwähnt—häufig eine geringe Hypertonie ihrer Körpersäfte gegenüber dem umgebenden Meerwasser erkennen lassen, vorzukommen. Sicher besitzen derartige Einrichtungen die *eurhalinen* Meeresvertebraten, wie z.B. *Carcinus maenas* und *Nereis diversicolor*. Wie oben gezeigt worden ist, sind diese Organismen in verdünntem Meerwasser aktiv homioosmotisch. Bei normaler Konzentration des Meerwassers sind bei ihnen Innen- und Aussenmedium isotonisch, bei Aufenthalt in verdünntem Seewasser überwiegt jedoch die Molarkonzentration der Körpersäfte die des Aussenmediums stets beträchtlich.

Bevor wir über den Sitz der osmoregulatorischen Einrichtungen bei diesen Tieren etwas aussagen, müssen wir zunächst die *Durchlässigkeit der Körperoberfläche* mariner Evertebraten ganz allgemein besprechen. Bekannt und von zahlreichen Autoren immer wieder festgestellt ist ja die Tatsache, dass stenohaline Meeresvertebraten in verdünntem Seewasser anschwellen und schwerer werden, in konzentriertem Meerwasser dagegen schrumpfen und an Gewicht verlieren. Man hat geglaubt hierin einen Beweis für eine Semipermeabilität der Körperoberflächen dieser Tiere zu sehen. Verschiedene Autoren haben diese Ansicht auf folgende Weise gestützt. Sie berechneten auf Grund der bekannten Konzentration und Menge der Körpersäfte, sowie der nach kürzerem oder längerem Aufenthalt in einem anisotonischen Medium beobachteten Gewichtsveränderung die endgültige Innenkonzentration unter der Annahme, dass nur reines Wasser ohne Salze durch die Haut permeiert sei und verglichen dann die berechneten mit den kryoskopisch ermittelten Werten. Auf diese Weise untersuchten Henri et Lalou (1904) verschiedene Seeigel und Bottazzi u. Enriques (1901) die Nacktschnecke *Aplysia*. Sie fanden übereinstimmend, dass die Membranen dieser Tiere in normalem, d.h. ungeschädigtem Zustand, semipermeabel waren. Wichtig erscheinen mir auch folgende Beobachtungen von Henri u. Lalou. Während Seeigel in einem Gemisch von Seewasser und Süßwasser (3:1) stets schwerer wurden, zeigten dieselben in einer isotonischen Lösung von Zucker in verdünntem Seewasser keinerlei Gewichtsveränderung während der ersten Stunden nach der Ueberführung in das Versuchsmedium. Der Chlorgehalt der Körperflüssigkeiten von Holothurien und Seeigeln veränderte sich in einer solchen Lösung während der ersten beiden Stunden ebenfalls nicht bemerkenswert, erst bei längerem Aufenthalt zeigte sich eine Abnahme des Cl-Gehaltes im Inneren und ein Eindringen nennenswerter Saccharosemengen. Alles dies weist auf eine Semipermeabilität der ungeschädigten Aussenmembranen hin. Auch Dekhuyzen (1920) kam auf Grund kurzfristiger Versuche an Sipunculiden zur gleichen Ansicht. Besonders einleuchtend erscheint mir folgender Versuch dieses Autors. Ein Exemplar von *Phascolosoma vulgare* von 3.125 g. Gewicht wurde nach Abspülen mit isotonischer Natriumnitratlösung für 5 Minuten in destilliertes Wasser überführt. Hierin stieg das Gewicht des Wurmes

auf 3·470 g. Während die Menge der Leibeshöhlenflüssigkeit um ca. 18·8 Prozent gestiegen war, zeigte das destillierte Wasser am Schluss des Versuches nur einen ganz geringen Cl-Gehalt (ca. 0·2 mg.). Es war demnach praktisch nur Wasser permeiert. Der Wurm wurde dann in normales Seewasser zurückgebracht und zeigte am nächsten Morgen fast genau sein ursprüngliches Gewicht, nämlich 3·127 g. Auch L. Frédéricq (1922) ist im allgemeinen der gleichen Ansicht. Er konnte zeigen, dass isotonische Lösungen (Zuckerwasser, etc.) die normalen Lebensfunktionen vieler mariner Evertebraten für einige Zeit zu erhalten vermögen. Andererseits glaubte er aber auf Grund langfristiger Versuche ein Eindringen körperfremder Stoffe (Zucker, Harnstoff) wahrscheinlich machen zu können; harnstoff- bzw. zuckerhaltiges Meerwasser erhielt nämlich die Lebenserscheinungen seiner Versuchstiere bzw. ihrer isolierten Organe (Herz von *Aplysia*, etc.) nur für wenige Stunden, bei längerer Versuchsdauer zeigten sich in allen Fällen Vergiftungsscheinungen. Ebenso gelang es ihm nach Zusatz geringer Mengen körperfremder Salze (Natriumnitrat, Ferrozyankalium) zum Meerwasser deren leicht nachweisbare Ionen nach einiger Zeit im Blut von *Carcinus maenas* aufzufinden. Frédéricq hält jedoch in diesem letzten Falle auch eine Resorption vom Darme aus für möglich. In gleicher Weise fand R. Quinton (1900, 1904) neben dem meist innerhalb kurzer Zeit erfolgenden osmotischen Wasseraustausch einen langsamen Transport von Salzen durch die Körperoberfläche (Austritt von Cl in hypotonischem, Aufnahme von Cl in hypertönischem Seewasser; Cl-Abgabe an isotonisches magnesiumpersulfathaltiges Seewasser). Ähnliche Versuche veröffentlichte neuerdings auch Bethe (1929). Er brachte Krebse (*Carcinus maenas*) und Nacktschnecken (*Aplysia punctata*) in künstliches Seewasser, dem ein Ion (Ca, K, Ng, Cl) fehlte oder im Überschuss zugesetzt war und fand, dass sich die Menge desselben Ions im Blute der Tiere in der gleichen Richtung änderte. Bethe ist der Ansicht, dass die so bewirkten Änderungen im internen Ionenmilieu eine Durchlässigkeit der Körperoberfläche für die betreffenden Ionen beweisen.

Den entscheidenden Beweis für seine Ansicht erbrachte Bethe vor kurzem (1930). Er wies nämlich nach, dass die Nacktschnecke *Aplysia* in einem Gemisch von Seewasser und isotonischer Zuckerlösung an Gewicht verliert (über 50 Prozent in 20 Stunden). Die einzige mögliche Erklärung dieser Beobachtung ist die: Der osmotische Druck ist innen und aussen gleich, die Salzkonzentration jedoch aussen geringer als innen. Da die Körperoberfläche permeabel für Salze ist, diffundieren dieselben nach aussen. Als Folge davon sinkt der osmotische Druck im Inneren, und es wird osmotisch Wasser nach aussen abgegeben. Das Volumen des Organismus und sein Gewicht nimmt ab!—Andere weichhäutige Meeresorganismen, wie Sipunculus und Holothurien, verhielten sich ähnlich; harthäutige Evertebraten, wie *Carcinus maenas*, zeigten nur eine Steigerung der Blutviscosität (thickening of the blood), dagegen keine Gewichtsabnahme.—*Die Körperoberflächen der marinen Evertebraten sind also sowohl für Wasser wie auch für Salze permeabel.* Da Wasser bedeutend schneller permeiert als die Salze, ist die Durchlässigkeit für die letzteren zumeist übersehen worden.—Eine Ausnahme erfährt diese Tatsache wahrscheinlich aber in den Fällen, in welchen der Ionengehalt des Blutes nicht mit dem des Meer-

wassers—bei gleicher Gesamtkonzentration—übereinstimmt (bei gewissen höheren Krebsen, die im Vergleich zu dem umgebenden Meerwasser mehr K und weniger Mg in ihrem Blut besitzen). Hier muss entweder eine Undurchlässigkeit der Körperoberfläche für die betreffenden Ionen bestehen oder ein regulatorischer Mechanismus vorhanden sein.

Weitere Untersuchungen über diese Frage erscheinen mir notwendig. Andererseits bin ich aber auch der Ansicht, dass man mit der Annahme einer einfachen Permeabilität die Eigenschaften der Haut der marinen Wirbellosen nur zum Teil erfasst. Vielleicht können Versuche von M. G. Dekhuyzen (1920) in dieser Hinsicht einen Schritt weiterbringen. Dieser Autor übertrug marine Würmer (*Phascolosoma vulgare*) in Meerwasser, das um 10 Prozent durch Zufügen von Süßwasser verdünnt war, bezw. in solches, das um 10 Prozent durch Zufügen von NaCl konzentriert war. Er bestimmte die Gewichtsveränderungen während der ersten 20 Minuten und fand, dass die Würmer im hypotonischen Medium weniger Wasser aufnahmen, als sie in den entsprechenden hypertonischen Medien verloren. Er möchte dieses Ergebnis durch die Annahme einer "défence" (osmotischen Resistenz?) von Seiten der Epidermiszellen in den hypotonischen Lösungen erklären. Wir werden weiter unten bei der Besprechung eigener Versuche (S. 333) zu einem ganz ähnlichen Ergebnis kommen.

Wir kommen jetzt zu der oben aufgeworfenen Frage nach dem Sitz der osmoregulatorischen Einrichtungen der euryhalinen, fakultativ homoiosmotischen Meeres-evertebraten (*Carcinus maenas*, etc.) zurück. Wir haben gesehen, dass diese Organismen in verdünntem Seewasser homoiosmotisch sind, dass sie also irgendwelche osmoregulatorische Einrichtungen besitzen müssen. Dementsprechend zeigt z.B. *Nereis diversicolor* nach Ueberführung in verdünntes Seewasser bei weitem nicht die grosse, durch Endosmose von Wasser bedingte Gewichtszunahme, wie etwa ein Vertreter der stenohalinen Meeresevertebraten (z.B. *Asterias rubens*) unter den gleichen Umständen. Nach Ueberführung aus Nordseewasser (35 v.T. Salzgehalt) in verdünntes Seewasser von 20 v.T. Salzgehalt nimmt *Nereis* innerhalb der ersten 3 Stunden über 20 Gewichtsprozent Wasser auf, schafft es dann aber im Laufe von 40 Stunden zum grössten Teile wieder hinaus (siehe Abb. 5). Ähnlich aber noch weit leistungsfähiger erweist sich *Carcinus maenas*. Sehr instruktiv erscheint mir ein schon an anderer Stelle veröffentlichter Versuche, den ich hier wiedergeben möchte:

Neun *Carcinus maenas* wurden aus Meerwasser von 32 v.T. Salzgehalt in solches von 20 v.T. Salzgehalt überführt. Fünf Tiere überstanden das Experiment gut und waren nach zweitägigem Aufenthalt in dem neuen Medium frisch und lebendig. Vier Tiere dagegen wurden nach einiger Zeit matt und starben nach ungefähr 20 Stunden. Sämtliche Krebse wurden von Zeit zu Zeit gewogen, um eine etwaige Wasseraufnahme festzustellen. Es zeigte sich, dass die fünf Tiere, die am Leben geblieben waren, in keiner Weise ihr Gewicht veränderten, während die vier anderen deutlich schwerer wurden (siehe Abb. 6). Gefrierpunktsbestimmungen, die nach einigen Tagen ausgeführt wurden, ergaben erwartungsgemäss bei den am Leben gebliebenen Tieren eine bedeutende Hypertonie der Körpersäfte gegenüber dem Außenmedium.

Dieser Versuch beweist, dass die Homoiosmie von *Carcinus* in verdünntem

Seewasser die Folge einer von dem lebenden Organismus geleisteten Arbeit ist. Ein kräftiges Individuum ist demzufolge nach Ueberführung in hypotonisches Seewasser imstande, eine osmotische Wasseraufnahme zu verhindern, während ein mattes, irgendwie geschwächtes Individuum eine solche Arbeitsleistung nicht mehr aufbringen kann. Auch auf andere Weise lässt sich diese Tatsache zeigen:

Eine Anzahl kräftiger Krebse wurde seit längerer Zeit in verdünntem Seewasser (20 v.T. Salzgehalt) gehalten und von Zeit zu Zeit gewogen. Ihr Gewicht blieb, wie zu erwarten, einigermassen konstant, ein einziges Exemplar wurde jedoch matt und starb nach einiger Zeit. Dieses Tier nahm auch als einziges an Gewicht—infolge osmotischer Wasseraufnahme—zu (Schlieper, 1929 c).

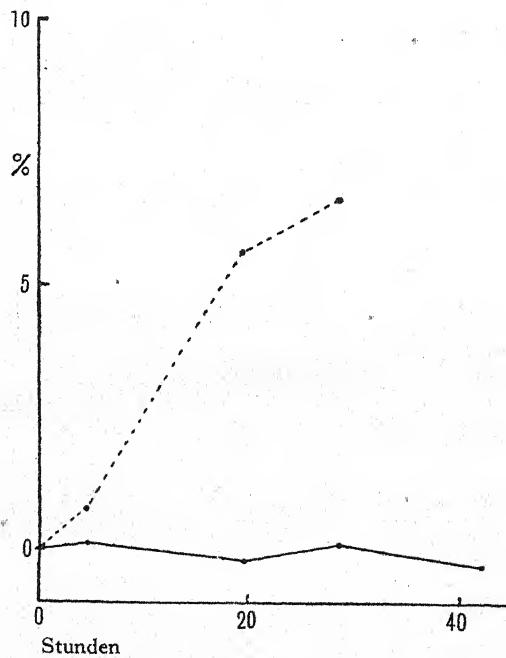


Abb. 6. Gewichtsänderung von *Carcinus maenas* nach Ueberführung aus Nordseewasser in verdünntes Seewasser von 20 v.T. Salzgehalt. Die ausgezogene Linie gibt die durchschnittlichen Gewichtsänderungen von 5 kräftigen Exemplaren an. Die gestrichelte Linie gibt diejenigen von 4 Exemplaren an, die im Verlaufe des Versuches matt wurden und starben.

Es liegt nun nahe, bei *Carcinus maenas* die Excretionsorgane (Antennendrüsen) als Sitz der osmoregulatorischen Mechanismen anzusprechen. Gewisse morphologische Untersuchungen an Süßwasserkrebsen weisen in die Richtung dieser Vermutung. Rogenhofer (1908) fand nämlich in Uebereinstimmung mit Grobben, u.a., dass die Süßwasserkrebse (Amphipoden, Isopoden, Dekapoden) fast allgemein längere Nephridialkanälchen als die verwandten marin Arten besitzen. Diese Tatsache spricht auf jeden Fall dafür, dass die Antennendrüsen der Süßwasserkrebsen osmoregulatorisch funktionieren. Für *Potamobius astacus* liess sich dieses

neuerdings auch beweisen (s. S. 338). Man sollte deshalb vielleicht erwarten, dass der bei *Carcinus* in verdünntem Seewasser produzierte Harn hypotonisch gegenüber dem Blut sein würde. Es ergab sich jedoch auf Grund von Gefrierpunktsbestimmungen, dass der Harn bei *Carcinus*, auch bei den in verdünntem Seewasser gehaltenen Exemplaren, mit dem Blut isotonisch ist.

Tabelle XVII. *Carcinus maenas* (nach Schlieper, 1929 c).

Aussen-medium Δ (°C.)	Blut Δ (°C.)	Harn Δ (°C.)	Zahl d. untersuchten Tiere
1.91	2.00	1.97	1
1.90	1.94	1.94	2
1.16	1.60	1.60	4
1.08	1.50	1.48	4
1.08	1.53	1.54	4
1.00	1.47	1.48	2
0.98	1.56	1.58	3
0.82	1.48	1.55	3
0.64	1.25	1.27	3

Die Excretionsorgane kommen also als Ursache der festgestellten Homioosmie nicht in Frage (auf die allgemeine Bedeutung dieses Befundes werden wir unten bei der Besprechung der osmoregulatorischen Einrichtungen der Süßwasser-wirbellosen noch eingehen). Auch der Darm kommt m.E. für eine derartige Funktion nicht in Betracht. Wir müssen deshalb annehmen, dass trotz der Hypotonie des Aussenmediums kein osmotischer Wassereinstrom stattfindet. Da aber die Haut, zumindest der Kiemen, sicher nicht wasserundurchlässig ist (Wasseraufnahme mattgewordener Krebse in hypotonischem Seewasser!) muss die Körperoberfläche bei *Carcinus* zu einer osmotischen Resistenz fähig sein, sie muss aktiv einen Wassereinstrom von aussen verhindern können. In Uebereinstimmung mit dieser Annahme kann man nach Zerstörung dieser osmotischen Resistenz durch Aetzen der Kiemenhaut mit Natronlauge eine beträchtliche, innerhalb ganz kurzer Zeit erfolgende Gewichtsvermehrung (Wasseraufnahme) beobachten (Schlieper).

Im Einzelnen ist aber hier noch Verschiedenes ungeklärt. Ich denke dabei besonders an die oben festgestellte Fähigkeit von *Carcinus*, sein Gewicht nach Ueberführung in ein hypotonisches Medium konstant zu erhalten, obgleich die Molarkonzentration seines Blutes in teilweiser Anpassung an das veränderte Aussenmedium beträchtlich abnimmt (s. Abb. 2 u. 6). Es gibt hierfür verschiedene Erklärungsmöglichkeiten. Einmal ist es denkbar, dass *Carcinus* nach Ueberführung in ein hypotonisches Medium so lange reines Wasser durch die Haut (osmotisch) aufnimmt, bis sich ein neuer Gleichgewichtszustand zwischen der Molarkonzentration des Blutes und der des Aussenmediums gebildet hat. Dadurch, dass nur so viel Wasser von aussen aufgenommen wird, als gleichzeitig Harn gebildet und hinausgeschafft wird, bleibt die Gewichtskonstanz trotz der Abnahme der inneren Molarkonzentration erhalten. Andererseits besteht aber auch die Möglichkeit, dass während des Anpassungsprozesses Salze durch die Haut (auf dem Wege der Diffusion) nach aussen an das hypotonische Medium abgegeben werden. Völlig ungelöst ist auch die Frage, auf welchem Wege *Carcinus* die für die Harnbildung benötigte Flüssigkeitsmenge aufnimmt. Es sind auch hier zwei Möglichkeiten denkbar, entweder eine Aufnahme durch die Haut oder eine solche durch den Darmtractus. Solange wir Ent-

gültiges hierüber nicht wissen, ist es auch unmöglich, den Mechanismus der Osmoregulation bei *Carcinus* voll zu verstehen.

Ganz ähnliche osmotische Leistungen wie bei *Carcinus* müssen wir bei all' den euryhalinen, marin Evertebraten voraussetzen, die in das Brackwasser der Flussmündungen oder sogar in reines Süßwasser vordringen. Namentlich in den Tropen können wir zahlreiche marine Tiere im Brack- und Süßwasser häufig beobachten. So sind z.B. nach Hesse (1924) die Süßwässer in der Umgebung des Golfes von Bengal, auf den Inseln des malayischen Archipels, in Madagaskar und im tropischen Amerika reich an aus dem Meere zugewanderten Bewohnern. "In Trinidad finden sich 18 km flussaufwärts, in völlig süßem Wasser—aber mit Ebbe- und Flutbewegung—an den Flussufern Bänke von *Mytilaceen*, im weichen Gestein bohrend die Bohrmuschel *Pholas* und andere Meerestiere." Auf den Inseln des indischen Archipels kommen *Palaemon carcinus* und *Varuna litterata* (dekapode Crustaceen) im Meer-, Brack-, und Süßwasser vor (M. Weber, 1892). In unseren Breiten wäre besonders *Mysis relicta* (ein Schizopode) zu nennen, die sowohl im Salzwasser der Ostsee als auch in gewissen norddeutschen Binnenseen zu finden ist. In der Ostsee kommen im Brackwasser des Bottnischen Meerbusens (4–6 v.T. Salzgehalt) noch folgende marine Evertebraten vor: 1 Ascidie, 4 Lamellibranchier, 1 Prosobranchier, 5 Amphipoden, 3 Isopoden, 1 Cirripedier, 1 Chaetopode, 1 Bryozoon und 1 Hydroide (Brandt, 1897). Man geht wohl nicht fehl in der Annahme, dass zumindest ein Teil dieser Organismen ein gegenüber dem Brackwasser ihres Fundortes hypertonicisches Innenmedium besitzt.—Weiterhin möchte ich an die klassischen Versuche von Beudant (1816) erinnern, dem es gelang, verschiedene marine Mollusken (*Mytilus*, *Ostrea*, *Cardium*, *Patella*, etc.) durch allmählichen Zusatz von Süßwasser zum Seewasser, in dem diese Tiere lebten, an reines Süßwasser zu gewöhnen und einige Zeit darin lebend zu erhalten. Ich führe diese Beispiele nur an, um zu zeigen, dass es doch recht zahlreiche marine Evertebraten gibt, die ähnlich wie *Carcinus* fakultativ homiosmotisch sind und dementsprechend ebenfalls zumindest zu einer osmotischen Resistenz befähigt sein müssen. Der einzige in dieser Richtung genauer untersuchte Wirbellose, der brachyure Dekapode *Eriocheir sinensis*, der sowohl im Süßwasser als auch im Meere anzutreffen ist, kommt seit einigen Jahren auch in Europa vor. Er ist in Deutschland an der Meeresküste (Elbmündung, Büsum) und auch im Binnenland (Oder, Havel) häufig (Schnakenbeck, 1926; Pax, 1929). Eine Fortpflanzung im Süßwasser ist bisher nicht beobachtet worden. Während bei Exemplaren, welche längere Zeit (mehrere Monate) in Süßwasser gehalten worden sind, die Gefrierpunkterniedrigung des Blutes $1\cdot09^{\circ}\text{C}$. (Mittelwert von 12 Tieren) beträgt, ist bei Seewassertieren eine annähernde Isotonie mit dem Außenmedium vorhanden. Führt man einen Krebs aus Süßwasser in Meerwasser über, so steigt die Molarkonzentration des Blutes langsam an, um nach einigen Tagen mit der des Außenmediums übereinzustimmen (s. Tab. XVIII). Aehnlich langsam geht die Anpassung von Salzwasserexemplaren an Süßwasser vorstatten. Bei Krebsen, die ich aus dem Hamburger Hafen erhielt und in fliessendes Süßwasser setzte, betrug der Gefrierpunkt des Blutes nach 4 Tagen noch $-1\cdot40^{\circ}\text{C}$., nach 9 Tagen $-1\cdot29$ und nach 14 Tagen noch $-1\cdot26^{\circ}\text{C}$.

Tabelle XVIII. *Eriocheir sinensis* nach Ueberführung aus Süßwasser
in Meerwasser.

(Schlieper, noch unveröffentlichte Versuche.)

Blut Δ (°C.)	Aussenmedium Δ (°C.)	Aufenthaltsdauer
1.16	2.12	0 Stunden
1.67	"	24 "
1.76	"	2 Tage
1.98	"	4 "
2.17	"	7 "

Der Wasseraustausch verläuft also bei *Eriocheir* im Gegensatz zu den andern marinen Evertebraten recht langsam. Während *Carcinus* schon nach 24 Stunden mit dem veränderten Aussenmedium im Gleichgewicht steht, dauert es bei *Eriocheir* stets mehrere Tage. Ich möchte annehmen, dass diese langsame Anpassung in Beziehung steht zu der Fähigkeit von *Eriocheir* grosse Schwankungen im Salzgehalt des Aussenmediums ohne Schaden zu überstehen. Wir wissen ja auf Grund zahlreicher experimenteller Untersuchungen, dass es gerade der schnelle Wasseraustausch ist, der die Evertebraten tötet bei der Ueberführung aus Meerwasser in ein weniger salzhaltiges Medium oder gar reines Süßwasser, bezw. umgekehrt. So sterben z.B. nach meinen Beobachtungen Seesterne (*Asterias rubens*), die man aus Nordseewasser (33 v.T. Salzgehalt) in zur Hälfte verdünntes Meerwasser überführt, unter Aufquellen (Wasseraufnahme) innerhalb weniger Stunden, während derselbe *Asterias* in der Kieler Förde (Ostsee) bei einem Salzgehalt von 15 v.T. in Mengen anzutreffen ist. Tiere, die wie *Eriocheir* einen solch schnellen Wasserwechsel nicht zeigen, ertragen auch die grössten Schwankungen im osmotischen Druck ihres Aussenmediums ohne Schaden. Ich werde auf diesen Punkt weiter unten bei der Bemerkung der Süßwasserteleostier (S. 341) noch einmal zurückkommen.

Wie hält nun *Eriocheir* die hohe Molarkonzentration seines Blutes im Süßwasser aufrecht? Die Untersuchung des Harnes ergab, dass derselbe sowohl bei Seewasser-, als auch bei Süßwassereemplaren annähernd die gleiche Konzentration wie das Blut besitzt. Bei Süßwassertieren ergab sich eine Gefrierpunkterniedrigung des Harnes von 1.10° C. (Mittelwert von 10 Bestimmungen); das Blut erstarrte bei annähernd derselben Temperatur, bei -1.09° C. (Mittelwert von 12 Bestimmungen). Die Excretionsorgane kommen also bei *Eriocheir* ebensowenig wie bei *Carcinus* für eine osmoregulatorische Funktion in Frage. Am wahrscheinlichsten ist auch hier wohl die Annahme, dass der Krebs sich im Süßwasser vollkommen vom Aussenmedium emanzipiert und keinerlei Wasser von aussen durch Endosmose aufnimmt, bezw. nur so viel, als er zur Harnbildung benötigt (Schlieper).

Anm.: In früheren Versuchen, die im Winter 1928/29 ausgeführt wurden, konnte ich beobachten, dass Exemplare deren Antennendrüsen verschlossen waren, in Süßwasser schnell an Gewicht zunahmen, während in gleicher Weise behandelte Seewassertiere diese Erscheinung nicht zeigten. Ich schloss hieraus auf eine von den Antennendrüsen aus-

geübte aktive osmoregulatorische Tätigkeit (Schlieper, 1929b). Im Sommer 1929, als ich die gleichen Versuche noch einmal wiederholte, zeigten jedoch dieselben Exemplare sowohl im Süßwasser als auch im Salzwasser nach Plombierung der Antennendrüsen eine übereinstimmende Gewichtszunahme. Dieses Ergebnis beweist, ebenso wie die oben mitgeteilten Harnuntersuchungen, dass der damals gezogene Schluss nicht zu recht besteht.

(3) Süßwasserevertebraten.

In die Physiologie des Wasserhaushaltes wirbelloser Süßwassertiere haben wir erst in neuerer Zeit einen Einblick erhalten. Einer der ersten, der über das Problem der Homioosmie der Süßwassertiere experimentiert hat, ist L. Frédéricq (1898). Er wies zuerst auf die ausserordentlich hohe Molarkonzentration im Innenmedium des Flusskrebses (*Potamobius astacus*) hin. Er dialysierte Flusskrebsblut in Pergamentpapierhülsen mit Leitungswasser und fand, dass die Gefrierpunkterniedrigung der so behandelten Blutproben rasch abnahm. Er zog daraus den Schluss, dass die Aufrechterhaltung des osmotischen Innendruckes beim Flusskrebs nicht auf einer Bindung der Salze an die Eiweiss-Substanzen des Blutes beruhe.

Einige Jahre später teilte Overton (1904) im Anschluss an seine klassischen Untersuchungen über die "Wasserökonomie" der Amphibien einige Beobachtungen an Wirbellosen mit. So stellte er fest, dass ein 5:31 g. schwerer Regenwurm innerhalb 62 Minuten durch Verdunstung 250 mg. Wasser verlor. Wurde ein solcher partiell ausgetrockneter Wurm mit seiner hinteren Hälfte in reines Wasser gesetzt, oder wurde der Wurm U-förmig gebogen und nur mit dem mittleren Teil in Wasser getaucht, so nahm er doch—durch die Haut—ziemlich schnell Wasser auf. Overton sprach auf Grund derartiger Versuche, die er mit dem gleichen Resultat auch an Schnecken wiederholte, die Ansicht aus, dass bei zahlreichen Wirbellosen des Süßwassers die Haut nach beiden Richtungen hin für Wasser durchlässig sei und stellte die lange Zeit allgemein anerkannte Hypothese auf, nach der bei diesen Organismen fortwährend Wasser durch die Haut osmotisch aufgenommen und durch besondere Excretionsorgane wieder ausgeschieden wird. Ohne die Tätigkeit der Excretionsorgane soll der osmotische Druck der Körpersäfte nicht aufrecht erhalten werden können.—Calugareanu (1914) fand, dass die Molarkonzentration der Körpersäfte von *Anodonta* bei längerem Hungern (80 Tage) stark abnimmt. Zur Erklärung dieser Beobachtung stellte er folgende, der Overtonschen Ansicht widersprechende Hypothese auf: Die Salze des Blutes sollen normalerweise durch die für sie permeable Haut in das Aussenmedium diffundieren und durch diejenigen, die in der Nahrung enthalten sind, ersetzt werden. Da aber während des Hungerns der Verlust der an das Aussenmedium abgegebenen Salze nicht kompensiert wird, sinkt der osmotische Druck in diesem Falle. Ein zwingender Beweis für diese Ansicht liegt allerdings nicht vor. Es wird nicht schwer fallen, sich auch andere Erklärungsmöglichkeiten auszudenken.—In guter Uebereinstimmung mit der Overtonschen Ansicht stehen dagegen einige Beobachtungen von Herfs (1922). Dieser Autor fand bei der Untersuchung des Wassergefäßsystems der Cercarien, dass die Entleerungsfrequenz der Endblase abhängig ist von der Molarkonzentration des Aussenmediums (s. Tab. XIX).

Tabelle XIX. Entleerungsfrequenz der Endblase des Wassergefäßsystems bei Cercarien (nach Herfs, 1922).

In Limneablut	Entleerung alle 15-7 Sek.
In Ringerlösung	" " 13.1 "
In 1/2 Ringerlösung	" " 8.3 "
In Süsswasser	" " 6.3 "

Es lässt sich also hier genau das Gleiche feststellen, wie bei den kontraktilen Vakuolen der Protozoen.—Auch bei Rotatorien wird der Inhalt des Excretionsystems durch rhythmische Pulsation des harnblasenartigen Endabschnittes entleert. Westblad (1922), der in neuester Zeit das Excretionssystem der Trematoden, Cestoden, Turbellarien und Rotatorien studiert hat, spricht die Ansicht aus, dass bei all diesen Tiergruppen—soweit sie im Süßerwasser bzw. in einem hypotonischen Medium vorkommen—das Wassergefäßsystem neben seiner mehr oder weniger entwickelten excretorischen Funktion in erster Linie als Regulator des Wassergehaltes im Körper dient. Aus der Blasenfrequenz und auf Grund von Messungen des Blasenvolumens berechnete er die Zeit, in der eine der Grösse des Körpervolumens entsprechende Wassermenge ausgeschieden wird (s. Tab. XX).

Tabelle XX. Leistung des Wassergefäßsystems bei Rotatorien und Trematoden (nach E. Westblad, 1922).

Art	Entleerungsfrequenz der Endblase	Verhältn. d. Blasenvolumens z. Körpervolumen	Zeit, in der eine d. Körpervolumen entspr. Wassermenge herausgeschafft wird
Trematoden <i>Distomum globiporum</i>	ca. 35 Sek.	ca. 1.70	1 Stunde 4 Min.
<i>Cercaria microcotyla</i>	1-3 Min.	ca. 1.40	2/3-2 Stunden
<i>Synchaeta tremula</i>	ca. 1 Min.	ca. 1.70	1 Stunde 10 Min.
Rotatorien <i>Ratulus</i> -Arten	3-4 Sek.	ca. 1.500	25-35 Min.

Mit der Annahme einer rein excretorischen Funktion scheinen diese gewaltigen Leistungen des Wassergefäßsystems nur schwer vereinbar. Westblad weist auch darauf hin, dass das Wassergefäßsystem bei den marinen Turbellarien entweder ganz fehlt oder nur sehr mangelhaft entwickelt ist. Die acoelen Turbellarien, die alle marin sind, besitzen keine Nephridien. Turbellariengattungen, welche sowohl marine als auch Süsswasser-Repräsentanten besitzen, zeigen bemerkenswerte Unterschiede in der Ausbildung ihres Wassergefäßsystems; bei den Süsswasserformen besitzt das stark entwickelte Wassergefäßsystem stets eine kontraktile Blase, bei den Salzwasserformen dagegen fehlt dieselbe, und das Wassergefäßsystem ist nur schwach ausgebildet. Alles dieses spricht für die osmoregulatorische Bedeutung dieses Organsystems.

Sehr unwahrscheinlich ist es, dass beim Regenwurm (*Lumbricus*) die Nephridien, deren Endblasen nur etwa alle drei Tage entleert werden, osmoregulatorisch wirken. Trotzdem können diese Würmer ohne Schaden für längere Zeit (Wochen) in

Wasser gehalten werden, ohne dass sich der osmotische Druck ihrer Körpersäfte ändert. Werden die Würmer in irgendwelche Salzlösungen (NaCl , Harnstoff, etc.) überführt, so steigt die Konzentration der Körpersäfte derart, dass stets ein Unterschied im osmotischen Druck zwischen Körperflüssigkeit und Aussenmedium aufrecht erhalten wird. Das alles beweist das Vorhandensein irgendwelcher osmoregulatorischer Mechanismen auch beim Regenwurm. Adolph (1926, 1927), dem wir einen Teil der geschilderten Beobachtungen verdanken, ist der Ansicht, dass die Haut in diesem Falle osmoregulatorisch funktioniert, ohne allerdings eine solche Funktion des Darmes mit Sicherheit ausschalten zu können.

Die Excretionsorgane der Süßwasserkrebse werden von verschiedenen Seiten für eine osmoregulatorische Funktion in Anspruch genommen. In erster Linie sprechen, wie bereits erwähnt, gewisse morphologische Untersuchungen hierfür. Rogenhofer (1905, 1908) fand in Uebereinstimmung mit Grobben u.a., dass die süßwasserlebenden Amphipoden, Isopoden und Decapoden fast allgemein längere Nephridialkanälchen als die verwandten marinen Arten besitzen. Dies spricht auf jeden Fall für eine grössere Arbeitsleistung von Seiten der Excretionsorgane der Süßwasserformen. Die Untersuchung des von den Antennendrüsen beim Flusskrebs (*Potamobius*) gebildeten Harnes bestätigte diese Ansicht. Der Harn ist bei *Potamobius* stets hypotonisch gegenüber dem Blut. Während das Blut bei -0.8°C . gefriert, erstarrt der Harn schon viel früher, bei *Potamobius leptodactylus* etwa bei -0.27°C . und bei *Potamobius astacus* schon bei -0.16°C . (nach Herrmann, mitgeteilt von Schlieper, 1929c). Es wäre aber vollkommen verfehlt, daraus den Schluss ziehen zu wollen, dass den Krebsen ohne eine derartige Arbeitsleistung von Seiten der Excretionsorgane ein Leben im Süßwasser ganz allgemein unmöglich wäre. Die oben mitgeteilten Untersuchungen an der chinesischen Wollhandkrabbe, *Eriocheir sinensis*, bei welcher stets Isotonie zwischen Harn- und Körperflüssigkeit festgestellt wurde, beweisen eindringlich das Gegenteil. Auch für einen Krebs, der ausschliesslich im Süßwasser vorkommt, für *Telphusa fluviatilis*, können wir das Gleiche zeigen. *Telphusa* nimmt unter den Süßwasserkrebsen insofern eine Ausnahmestellung ein, als bei ihr in der Grösse der Excretionsorgane kein Unterschied gegenüber den im Meere lebenden Verwandten zu konstatieren ist (Marchal, 1892). Gefrierpunktsbestimmungen des Harnes bei diesem in Italien häufigen Krebs ergaben eine Erklärung für diese auffallende Erscheinung. Der von den Antennendrüsen produzierte Harn ist nämlich isotonisch zum Blut (s. Tab. XXI).

Tabelle XXI. *Telphusa fluviatilis* (Schlieper, noch unveröffentlichte Versuche).

Harn Δ (°C.)	Blut Δ (°C.)	Bemerkung
1.20	1.18	Harn- u. Blutuntersuchungen wurden
1.12	1.13	nicht an den gleichen Tieren vorgenommen
1.18	1.17	

Hierdurch ist die Einfachheit im Bau der Excretionsorgane bei *Telphusa* ohne weiteres erklärt; sie funktionieren ebenso wie bei den marinen Krebsen rein excre-

torisch. Trotzdem müssen aber auch bei *Telphusa* irgendwelche osmoregulatorischen Mechanismen existieren, die dahin wirken, dass ein Unterschied in der Konzentration zwischen Innen- und Aussenmedium dauernd erhalten bleibt. Setzt man nämlich ein Exemplar in blutisotonisches Meerwasser, so steigt die Konzentration des Blutes über das normale Mass hinaus (s. Abb. 3, S. 319). Ich möchte annehmen, dass es auch hier die Haut ist, welche osmotische Arbeit leistet.

Mit der Tatsache, dass im Süßwasser auch Tiere existieren können, deren Excretionsorgane nicht osmoregulatorisch funktionieren, steht gut im Einklang das Vorkommen solcher Tierformen im Süßwasser, die überhaupt keine besonders ausgebildeten Nierenorgane besitzen. Ich denke dabei besonders an die Süßwasserschwämme und die Hydrozoen (*Hydra*, *Cordylophora*, *Limnocnida*, etc.), die sich an das Leben im Süßwasser angepasst haben; auch die in das freie Wasser abgelegten Eier der Süßwassertiere gehören hierher. Auf Grund der Hypothese von der "Schutzfunktion der Excretionsorgane gegen Aussüssung" ist das Vorkommen dieser dünnhäutigen und durchlässigen Formen im Süßwasser nicht erklärbar. Selbst Herfs (1922), der doch ganz auf dem Boden der Overton'schen Hypothese steht, schreibt: "Die Anpassung dieser scheinbar so ganz ungeschützten und dem Aussüßen völlig preisgegebenen Tierformen an das Süßwasser, erscheint deshalb so rätselhaft, weil bisher keinerlei Vorrichtungen zur Beseitigung des eindiffundierenden Wassers gefunden sind." Verständlich ist diese Tatsache jetzt erst, wo wir wissen, dass auch verschiedene höhere Tierformen des Süßwassers (*Telphusa*, *Eriochair*) ohne ein solches wasserhinausschaffendes Nierenorgan existieren können, bei denen also von einem dauernden umfangreichen Wasser-durchstrom keine Rede sein kann. Nicht die Nierenorgane, wahrscheinlich auch nicht der Darm, sondern die an das Aussenmedium angrenzenden Hautschichten der Süßwasserwirbellosen sind die Ursache der Homioosmie dieser Tierformen.

Unberührt von dieser Feststellung bleibt aber ein anderes Problem: die Frage nach der Bedeutung des dauernden intensiven Wasserdurchstromes bei vielen Süßwasserwirbellosen. Ludwig (1928) nimmt auf Grund umfangreicher Berechnungen an, dass er bei ciliaten Infusorien (*Paramaecium*) der Atmung, speziell der Ausscheidung von Kohlensäure, diene. Weitere Untersuchungen sind auch hier notwendig.

(4) Selachier.

Bei den marinen Selachiern ist die Molarkonzentration der Körpersäfte, wie oben erwähnt, um ein Geringes höher als die des Aussenmediums. Der hohe osmotische Druck des Innenmediums kommt nur durch die Anreicherung des Blutes mit einem endogenen Stoffwechselprodukt, dem Harnstoff, zustande. Der Gehalt an anorganischen Salzen ist ebenso wie bei den marinen Teleostieren wesentlich geringer als der des umgebenden Meerwassers (Squaliden 38 Prozent, Rochen 48 Prozent von dem des Meerwassers). Während die inneren Gewebe und vor allem die Blutkörperchen für Harnstoff durchlässig sind, ist dieses bei den Aussenmembranen, insbesondere den Kiemen, nicht der Fall (Duval, 1925). Auf diese Weise ist die—wenn ich im Anschluss an Duval so sagen darf—physiologische

Molarkonzentration im Blute der Selachier ähnlich niedrig wie bei den marinischen Knochenfischen, nur mit dem Unterschied, dass es bei den Selachiern keiner besonderen osmotischen Arbeitsleistung dazu bedarf, da ja annähernde Isotonie zwischen Innen- und Aussenmedium besteht. Der Harn der Selachier zeigt ungefähr die gleiche Molarkonzentration wie das Blut. Bottazzi (1911) gibt für *Scyllium stellare* für das Blut einen Gefrierpunkt von -2.23°C . an und für den Harn einen solchen von -2.40°C . Die bisherigen spärlichen Untersuchungen haben, wie oben gezeigt, eine weitgehende Abhängigkeit der Molarkonzentration der Körpersäfte von der des Aussenmediums erwiesen. Trotzdem müssen irgendwelche osmoregulatorische Einrichtungen bestehen, das beweist die geringe Hypertonie der Körpersäfte gegenüber dem Aussenmedium bei den marinischen Selachiern, und das verlangt das Vorkommen von 22 Selachierarten in tropischen Süßwässern (Hesse, 1924). Leider gibt es hierüber keinerlei Untersuchungen.

(5) Stenohaline Meeresteleostier.

Ueber die Osmoregulation der marinischen Knochenfische können wir nur wenig Positives aussagen¹. Nach den Untersuchungen von Sumner und Gueyland tritt nach Ueberführung in verdünntes Seewasser eine Gewichtszunahme (Wasser- aufnahme) ein. Die Konzentration des Harnes ist stets geringer als die des Blutes (s. Tab. XXII).

Tabelle XXII (aus Bottazzi, 1911).

Art	Blut Δ ($^{\circ}\text{C}.$)	Harn Δ ($^{\circ}\text{C}.$)
<i>Lophius piscatorius</i>	1.040	0.775
" "	0.978	0.706
" "	0.770	0.643
<i>Conger vulgaris</i>	1.025	0.820
<i>Gadus morrhua</i>	0.652	0.644
<i>Anarrhichas lupus</i>	0.681	0.555
<i>Cottus scorpius</i>	1.159	0.764

Die Nieren können demnach nicht der Sitz der osmoregulatorischen Mechanismen sein. Warum schrumpfen nun aber die Teleostier nicht bis zum Druckausgleich? Man hat zur Erklärung dieser Tatsache verschiedene Hypothesen aufgestellt:

(1) Der Ueberschuss an Salzen wird nicht durch die Nieren sondern durch ein anderes Organ ausgeschieden.

(2) Der Harn wird durch das Wasser verdünnt, das sich durch Verbrennung im Organismus bildet.

(3) Die Haut transportiert aktiv Wasser in das Innere.

Ueber diese Formulierung der Probleme ist man in den letzten Jahren nicht hinausgekommen.

(6) Stenohaline Süßwasserteleostier.

Auch über die osmoregulatorischen Mechanismen dieser Tiergruppe existieren kaum irgendwelche Untersuchungen. Sie erscheinen aktiv homoiosmotisch im

¹ Die Körpersäfte sind hypotonisch gegenüber dem Aussenmedium (s. Tab. I).

Süßwasser, dagegen passiv poikilosmotisch im Salzwasser (s. oben, S. 320). Nach Ueberführung in Salzwasser tritt eine Gewichtsabnahme (osmotische Wasserabgabe) ein.

(7) *Euryhaline Teleostier.*

Die euryhalinen Teleostier, die, wie z.B. der Aal (*Anguilla anguilla*) und der Lachs (*Salmo salar*), sowohl im Salzwasser als auch im Süßwasser leben können, sind beiden Medien gegenüber aktiv homoiosmotisch. Sie besitzen also vereinigt die osmoregulatorischen Fähigkeiten der Meeres- und der Süßwasserteleostier. Wenn auch von einem vollkommenen Verständnis der osmoregulatorischen Mechanismen dieser Tiere noch keine Rede sein kann, so haben wir doch in den letzten Jahren verschiedene interessante Einblicke erhalten. So wissen wir z.B., dass der die Haut beim Aal bedeckende Schleim eine wichtige osmotische Schutzhülle darstellt, ohne welche der Aal eine Ueberführung aus Süßwasser in Seewasser nicht lebend übersteht (P. Bert). Duval (1925) hat diese Erscheinung genauer untersucht und gefunden, dass bei einem Exemplar, welches seiner Schleimhülle beraubt worden ist, nach Ueberführung in Salzwasser ein bedeutend stärkerer osmotischer Wasser-austausch auftritt, als bei einem solchen, dessen Schleimhülle unverletzt ist (s. Tab. XXIII).

Tabelle XXIII. *Anguilla anguilla* (nach Duval, 1925).

(1) Versuchs-medium Δ (°C.)	(2) Serum d. Aales ohne Schleimhülle Δ (°C.)	(3) Serum d. Aales mit Schleimhülle Δ (°C.)	(4) Differenz zwisch. z. u. 3. (°C.)
0.02	0.58	0.62	- 0.04
0.68	0.76	0.68	+ 0.08
0.83	0.79	0.69	+ 0.10
1.13	0.93	0.71	+ 0.22
1.57	1.21	0.74	+ 0.47
2.13	1.15	0.79	+ 0.36

Der auf der Haut liegende Schleim schützt also den Aal vor einer zu schnellen grösseren Änderung im osmotischen Druck seiner Körpersäfte, welche die meisten übrigen Süßwassertiere nach der Ueberführung in Seewasser töten. Ich möchte darauf hinweisen, dass der Süßwasserkrebs *Telphusa fluvialis*, der eine plötzliche Ueberführung aus Süß- in Seewasser ohne weiteres erträgt, ebenfalls eine ganz langsame (mehrere Tage währende) Anpassung an den erhöhten Salzgehalts des Aussenmediums zeigt (Duval).

Ganz neue Bahnen beschritt Gueylard (1923) bei der Untersuchung der osmoregulatorischen Einrichtungen des Stichlings (*Gasterosteus*). Schon ältere Autoren (P. Bert, Milne Edwards, Semper, Florentin, Giard) hatten beobachtet, dass dieser kleine im Süßwasser häufige Teleostier eine plötzliche Ueberführung in Seewasser mehr oder weniger gut verträgt. Während aber P. Bert und Milne Edwards ihre Versuchstiere 1-2 Monate in Seewasser lebendig erhalten konnten, gelang dies Florentin nur für wenige Stunden. Gueylard versuchte deshalb zunächst diese widersprechenden Befunde aufzuklären und prüfte zu diesem Zweck

den Einfluss der verschiedensten Faktoren auf die Zeitdauer des Ueberlebens (Temperatur, Jahreszeit, chemische Zusammensetzung und Wasserstoffionenkonzentration des Aussenmediums, etc.). Sie stellte dabei zunächst fest, dass die einzelnen Arten des Stichlings ganz verschieden widerstandsfähig gegen Seewasser sind. Während *Gasterosteus pungitius* in Salzwasser ($\Delta = 1.33^\circ \text{C}$) im Durchschnitt nur 12 Stunden am Leben blieb, starb *Gasterosteus leiuрус* erst nach achtätigem Aufenthalt in dem gleichen Medium. Für ihre weiteren Versuche benutzte deshalb die Autorin nur die widerstandsfähigere Art. Sie fand dabei, dass vor allem der Jahreszeit ein wesentlicher Einfluss zukommt. Während z.B. ein Stichling im Januar (in Seewasser, $\Delta = 2.16^\circ \text{C}$) 16 Tage am Leben blieb, trat der Tod eines anderen Exemplares im Mai bereits nach 48 Minuten ein. Dann wirkte auch eine reine Salzlösung (NaCl) bedeutend giftiger als äquilibrierte Lösungen, wie z.B. das Seewasser eine darstellt. Weiterhin konnte sie feststellen, dass ein plötzlicher Wechsel in der

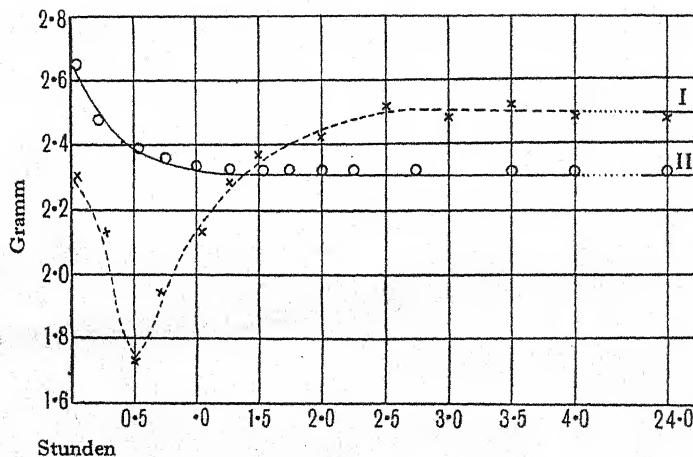


Abb. 7. Gewichtsänderungen zweier Süßwasserfische nach Ueberführung aus Süßwasser in Salzwasser. I. - - - = *Gasterosteus leiuucus* nach Ueberführung in Meerwasser ($\Delta = 2.02^\circ \text{C}$); II. — = *Gobio fluviatilis* nach Ueberführung in verdünntes Seewasser ($\Delta = 0.65^\circ \text{C}$) (nach Gueylard).

Konzentration des Aussenmediums in ganz typischer Weise das Gewicht dieser Fische beeinflusst. Sie studierte diese Gewichtsveränderungen, um einen Einblick in den osmotischen Wasseraustausch des Stichlings nach Ueberführung in ein Medium von verändertem Salzgehalt zu erhalten und machte dabei eine ganz unerwartete Feststellung. Nach Ueberführung eines Stichlings in Seewasser nahm das Gewicht zunächst—in Laufe der ersten 30 Minuten—rapid ab, um dann schnell wieder anzusteigen und übertraf schliesslich nach einigen Stunden sogar den Anfangswert. Das Gleiche, umgekehrt, liess sich nach Ueberführung eines an Seewasser angepassten Exemplares in reines Süßwasser beobachten: zuerst eine schnelle Gewichtszunahme, dann ein reversibler Prozess, der die anfängliche Gewichtsänderung wieder rückgängig macht, sogar überkompensiert, derart, dass das endgültige Gewicht unter dem Ausgangswert zu liegen kommt (s. Abb. 7). Steno-

haline Teleostier (der Gründling *Gobio fluviatilis*) zeigten nichts Derartiges, bei ihnen verlief die Gewichtskurve stets gleichmässig in ein und derselben Richtung. Das Gewicht am Schluss des Versuches war stets geringer als das Anfangsgewicht, wenn die Konzentration des Aussenmediums erhöht wurde; es war dagegen grösser, wenn der Salzgehalt im Aussenmedium vermindert wurde (s. Abb. 7). Die anfängliche Gewichtsänderung scheint beim Stichling ebenso wie bei den stenohalinen Teleostieren rein passiv durch die osmotische Wirkung des Aussenmediums bedingt zu sein, dann muss aber bei Eintritt des reversiblen Prozesses ein regulatorischer Mechanismus in Tätigkeit treten, der den stenohalinen Teleostieren fehlt. Messungen des Gesamtwassergehaltes und der Molarkonzentration der durch Auspressen aus den Geweben erhaltenen Körpersäfte stehen in guter Uebereinstimmung mit den oben geschilderten Ergebnissen. Während bei den stenohalinen Teleostieren der Gesamtwassergehalt abnahm, und die Molarkonzentration der Pressäfte stieg, wenn der Salzgehalt im Aussenmedium erhöht wurde, blieben beim Stichling beide Werte unter den gleichen Bedingungen unverändert.

Wie stellt sich nun F. Gueylard den beim Stichling wirksamen osmoregulatorischen Mechanismus vor? Sie nimmt an, dass das Verhältnis vom Cholesterinzum Gesamtfettsäurengehalt im Blut ("coefficient lipocytique"), welches nach den Untersuchungen von Mayer und Schaeffer das Imbibitionsvermögen der Gewebe bestimmt, eine wesentliche Rolle spielt. Die Regulation von Seiten des Stichlings, die sich in der rückläufigen Gewichtsveränderung nach der anfänglichen Gewichtsabnahme in Seewasser ausdrückt, soll dadurch zustande kommen, dass der Cholesteringehalt im Blut und in den Geweben aktiv erhöht wird, wodurch eine weitere Wasserabgabe der Gewebe verhindert und vielleicht sogar das Imbibitionsvermögen gesteigert wird. Die Bildung der hierzu notwendigen Cholesterinmengen soll in der Milz erfolgen. Gewisse Anzeichen sprechen nun tatsächlich für die Richtigkeit dieser Hypothese, andere jedoch ebenso stark gegen sie. So fand Gueylard den Cholesteringehalt bei in Salzwasser gehaltenen Stichlingen tatsächlich grösser als bei Süsswasserexemplaren. Stenohaline Teleostier zeigten dagegen diesen Unterschied nicht. Auch soll das Gewicht der Milz bezogen auf das Gesamtgewicht des Körpers beim Stichling grösser sein als bei den stenohalinen Teleostieren. Führt man einen Stichling ins Seewasser über, so nimmt das Gewicht der Milz deutlich ab; bei den anderen Teleostieren ist etwas Derartiges nicht zu bemerken.—Das alles spricht für die Anschauungen von F. Gueylard. Dagegen spricht aber folgende eigenartige Tatsache, auf die auch Duval hinweist: Der mit gewissen Chemikalien (Chloroform, Aether, Azeton, etc.) getötete Stichling zeigt nach Gueylard die gleichen Gewichtsveränderungen bei Ueberführung in Seewasser wie der lebende! Der osmoregulatorische Mechanismus braucht also durch den Tod des Fisches nicht unbedingt zerstört zu werden. Ist diese Tatsache mit der obigen Hypothese vereinbar? Wie wird bei dem toten Fisch das in der Milz vorhandene Cholesterin zu den Geweben transportiert, da der Blutkreislauf doch stillsteht?—Auch der Aal passt sich den oben skizzierten Anschauungen nicht ein. Einerseits entspricht bei ihm das Gewicht der Milz ganz den bei stenohalinen Teleostieren gefundenen Grössenwerten, dann sind aber auch keine eindeutigen

Unterschiede im Gewicht der Milz bei Seewasser- und Süsswasserexemplaren zu konstatieren. Schliesslich lebt nach Gueylard ein Aal, dessen Milz operativ entfernt worden ist, in Seewasser genau so lang wie in Süsswasser. Ob nun beim Aal andersgeartete osmoregulatorische Mechanismen (Schleimhülle, etc.!) vorhanden sind, oder ob die angegebenen Tatsachen ausreichen, um die Gueylardsche Hypothese überhaupt zu widerlegen, möchte ich nicht entscheiden. Sicher sind die von F. Gueylard ausgesprochenen Gedanken zumindest in dem Sinne wertvoll, als sie zu weiteren Untersuchungen anregen und ganz neue Bahnen weisen.

(8) Amphibien.

Ueber den Wasserhaushalt der Amphibien wissen wir Dank zahlreicher Untersuchungen recht genau Bescheid. Die Frösche sind, wie wir oben gesehen haben, im Süsswasser homiosmotisch, in Salzwässern dagegen passiv poikilosmotisch, d.h. sie sind in hypertonischen Salzlösungen nicht imstande, den bei Aufenthalt in Süsswasser vorhandenen osmotischen Eigendruck ihrer Körpersäfte aufrecht zu erhalten. Overton (1904) untersuchte die Durchlässigkeit der Froschhaut und fand u.a. Folgendes: Wurde z.B. ein ursprünglich 0·6 g. schwerer Laubfrosch (*Hyla arborea*), der durch Verdunstung in trockener Luft ca. 2 g. Wasser verloren hatte, bis zu den Vorderbeinen in reines Wasser getaucht, so dass er nicht trinken konnte, so nahm er bei einer Wassertemperatur von 20° C. innerhalb 10 Minuten = 0·9-1·0 g. Wasser durch die Haut auf.—Wurde ein Frosch mit zugebundener Kloake in reines Wasser gesetzt, so dass er nicht trinken konnte, dann sammelte sich das durch die Haut aufgenommene und vermittels der Nieren als sehr verdünnter Harn ausgeschiedene Wasser in der Kloake und im Darmkanal und bewirkte so eine bedeutende Gewichtszunahme. In Kochsalzlösungen (0·8 Prozent u. mehr) verloren die Frösche dagegen regelmässig an Gewicht; sie gaben so lange Wasser durch die Haut ab, bis ihr Blut annähernd den gleichen osmotischen Druck zeigte wie die äussere Salzlösung.—Befand sich ein Frosch so in einer 0·8-0·9 prozentigen KCl-Lösung, so dass sein Kopf ausserhalb der Lösung blieb und er nicht trinken konnte, so traten selbst bei wochenlang dauernden Versuchen keine Vergiftungsscheinungen auf, obgleich schon ein Gehalt des Blutes von 0·1 Prozent KCl sofort zu den schwersten Lähmungen führt.—Overton nahm auf Grund dieser und ähnlicher Versuche an, dass die Haut der Amphibien impermeabel für Salze sei, dagegen nach beiden Richtungen hin durchlässig für Wasser. Die Osmoregulation sollte in Uebereinstimmung hiermit in Süsswasser so vor sich gehen, dass das durch die Haut osmotisch aufgenommene Wasser, durch fortgesetzte Nierentätigkeit wieder ausgeschieden würde. Gefrierpunktsbestimmungen des Harnes, die eine sehr niedrige Konzentration desselben erkennen liessen, schienen diese Ansicht zu bestätigen. Ebenso sprach dafür die Tatsache, dass in isotonischen bzw. schwach hypertonischen Salzlösungen nur geringe Mengen eines Harnes produziert werden, dessen Molarkonzentration ungefähr der des Aussenmediums entspricht (s. Tab. XXIV).

Neuere Untersuchungen von Parnas (1921), Przylecki (1922-4), Wertheimer

Tabelle XXIV. *Rana esculenta*.

Aussenmedium Δ (°C.)	Blut Δ (°C.)	Harn Δ (°C.)	Autor
0·01	0·435	0·170	Bottazzi
0·610	0·640	0·620	Brunazzi
0·700	0·740	0·695	"
0·765	0·750	0·845	"

(1923-4), Bauer (1925) und Adolph (1925-7) haben jedoch ergeben, dass die osmoregulatorischen Mechanismen bei den Amphibien in ganz anderer Weise arbeiten, als man sich bisher vorgestellt hatte. Przylecki zeigte, dass die Haut des Frosches wohl für Salze durchlässig ist, dass aber das Ausbleiben einer Vergiftung in isotonischen KCl-Lösungen daran liegt, dass das durch die Haut langsam eindringende Salz stets sofort durch die Nierentätigkeit wieder aus dem Organismus herausgeschafft wird, sodass niemals die Konzentration im Blute so gross werden kann, dass Lähmungen entstehen. Dann konnten Wertheimer, Przylecki und Bauer—zum Teil in Wiederholung älterer Versuche von Reid und Maxwell—zeigen, dass die lebende Froschhaut auch dann einen Wassertransport aufweist, wenn sie als Membran zwischen zwei isotonische Kochsalzlösungen geschaltet wird; in diesem Falle können keinerlei osmotische Kräfte als Ursache für den Wassertransport in Frage kommen und doch findet ein solcher in der Richtung von aussen nach innen statt. Anscheinend kann auch die Stärke dieses Wassertransports von Seiten der Gewebe irgendwie reguliert werden, bindet man nämlich die Ureteren oder die zuführenden Nierengefäße ab, so nimmt der Wassereinstrom, ohne dass der Gefrierpunkt des Blutes und damit das Konzentrationsgefälle zwischen Innen- und Aussenmedium wesentlich verändert ist, allmählich ab (Przylecki). In neuester Zeit hat sich besonders E. F. Adolph in zahlreichen Arbeiten um die Erforschung des Wasserhaushaltes der Amphibien verdient gemacht. Er zeigte, dass beim Frosch die Menge des durch die Nieren herausgeschafften Wassers von der Konzentration des Aussenmediums, solange dasselbe hypotonisch ist, nicht wesentlich beeinflusst wird. In einer m/10 NaCl-Lösung wird nicht weniger Harn produziert als in Süsswasser, erst in viel höher konzentrierten Medien nimmt die gebildete Harnmenge ab (s. Abb. 8). Die Intensität des Wassereinstromes ist also von der Differenz zwischen Aussen- und Innénmedium nicht ohne weiteres abhängig. Ebenso ist auch die Grösse der Wasserexcretion bei verschiedenen grossen Individuen nicht der Körperoberfläche sondern dem Gewicht proportional. Die Tätigkeit der Nieren richtet sich auch durchaus nicht immer nach der Intensität des Wassereinstroms; nur so ist es zu verstehen, wenn bei hohen Temperaturen infolge einer Steigerung der Wasserexcretion das Körpergewicht sinkt, während bei niedrigen Temperaturen eine Gewichtszunahme zu beobachten ist. Normalerweise aber entspricht die Grösse des Wassereinstromes der Menge des durch die Nieren hinausgeschafften Wassers.

Zusammenfassend müssen wir also betonen, dass resorbierende Haut und ausscheidende Niere den Wasserdurchstrom und den Wasserhaushalt regulieren,

und dass der aktiv geleitete Durchstrom anscheinend über dem osmotischen Einstrom dominiert (Parnas).

Anhangsweise möchte ich schliesslich noch erwähnen, dass sich die osmoregulatorischen Mechanismen während der Entwicklung der Amphibien in interessanter

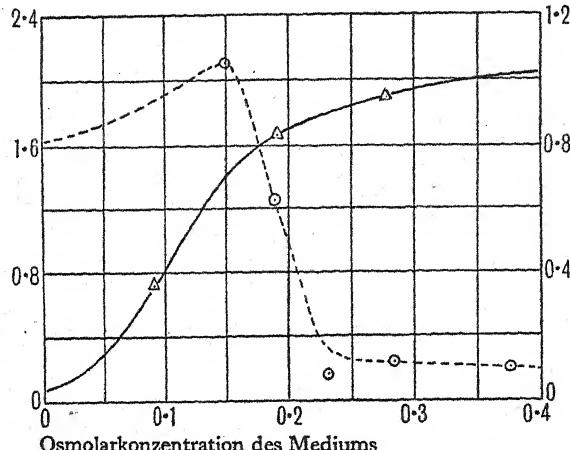


Abb. 8. *Rana pipiens*. Wasser- und Chloridausscheidung durch die Nieren, in Prozenten des Körpergewichtes pro Stunde, bei Aufenthalt in Kochsalzlösungen verschiedener Konzentration (nach Adolph). - - - = Wasserausscheidung. — = Chloridausscheidung.

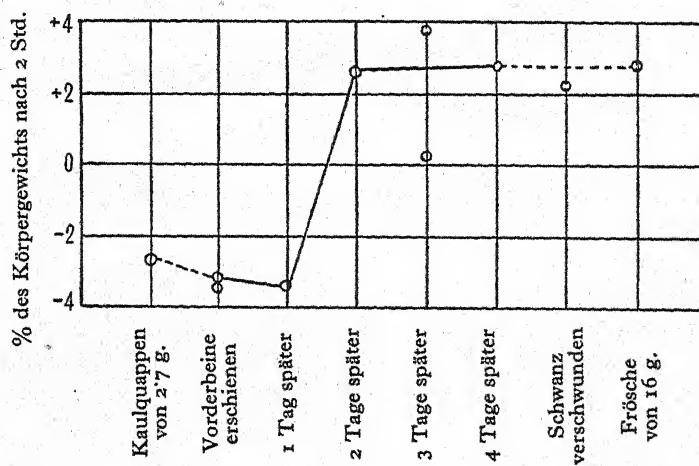


Abb. 9. *Rana pipiens*. Gewichtsänderungen verschiedenaltriger Frösche in 0.08 mol. NaCl-Lösungen während der Metamorphose. Die Gewichtsänderungen sind auf solche bezogen, welche Frösche gleichen Alters in Leitungswasser erleiden (nach Adolph).

Weise ändern. Es lässt sich nämlich zeigen, dass die Kaulquappen von *Rana pipiens* in verdünntem 0.08 molaren NaCl-Lösungen an Gewicht verlieren, während junge Frösche nach der Metamorphose trotz unveränderter Molarkonzentration der Körpersäfte in dem gleichen Medium eine Gewichtszunahme erfahren (s. Abb. 9). Adolph (1927), dem wir diese Beobachtung verdanken, nimmt an,

dass dieser Wechsel in der Osmoregulation in Beziehung steht zu gleichzeitig auftretenden Aenderungen in der morphologischen Differenzierung der Haut.

VI. ATMUNG UND OSMOREGULATION.

Die aktive Osmoregulation der homiosmotischen Wassertiere ist notwendigerweise ebenso wie jede Arbeitsleistung des lebendigen Organismus von einem Energieverbrauch begleitet. Da bekanntlich die arbeitende Niere ebenso wie der resorbierende Säugetierdarm einen stark erhöhten Sauerstoffverbrauch aufweist, lag es nahe, auch in diesem Falle die Energiequelle in den Oxydationen der lebenden Zelle zu suchen. Neuere Versuche scheinen wenigstens in gewissen Fällen diese Ansicht zu bestätigen. So fand Schlieper (1929a), dass bei *Carcinus maenas* der Sauerstoffverbrauch mit abnehmender Salzkonzentration im Aussenmedium zunimmt (s. Tab. XXV).

Tabelle XXV. *Carcinus maenas* (nach Schlieper, 1929a).

Salzgehalt (v.T.)	O ₂ -Verbrauch
16	96
10	109
7	118

Auch die Untersuchung der Atmung isolierter Kiemen von *Mytilus edulis* ergab Aehnliches. Es wurden frische Miesmuscheln, die aus der Kieler Förde (16 v.T. Salzgehalt) stammten, in verdünntes Seewasser (6 v.T. Salzgehalt) überführt; nach längerer oder kürzerer Zeit wurden die Kiemen herausgeschnitten und der Sauerstoffverbrauch derselben gemessen (s. Tab. XXVI).

Tabelle XXVI. Sauerstoffverbrauch isolierter *Mytilus*-Kiemen nach Ueberführung in hypotonisches Seewasser (n. Schlieper, 1929a).

Sauerstoffverbrauch pro 100 mg. Trocken- substanz (ccm.)	Salzgehalt des Versuchsmediums (v.T.)	Aufenthaltsdauer der Muscheln in dem Ver- suchsmedium vor Heraus- schneiden der Kiemen
0.307	16	—
0.368	6	6 Stunden
0.344	6	"
0.305	6	27 "

Die Atmung der isolierten Kiemen war also wenigstens für einige Zeit nach der Ueberführung in hypotonisches Seewasser stärker geworden. Ich möchte diese zeitweise Steigerung des Sauerstoffverbrauches als Zeichen einer aktiven von Seiten der Kiemenzellen ausgeübten osmotischen Resistenz ansehen, die allerdings auf die Dauer nicht imstande zu sein scheint, eine Angleichung an das Aussenmedium zu verhindern, daher wohl die Abnahme des Sauerstoffverbrauches nach einiger Zeit. Versuche von Beudant (1816), nach denen *Mytilus edulis* selbst in reinem Süßwasser für einige Zeit lebensfähig ist, sind geeignet, diese Ansicht zu

unterstützen.—Gegenüber hypertonischen Lösungen verhalten sich auch die euryhalinen Evertebraten (z.B. *Carcinus* nach Frédéricq) poikilosmotisch; dementsprechend kann man bei *Mytilus* nach Ueberführung in hypotonisches Seewasser keinerlei Steigerung der Oxydationsprozesse, sondern im Gegenteil eine Abnahme derselben beobachten, die ich auf eine Schädigung durch osmotischen Wasserentzug zurückführen möchte.

Tarussov (1927) fand in kurzfristigen Versuchen bei *Nereis diversicolor* eine Steigerung der Atmung in hypotonischen Medien, eine Abnahme dagegen bei Hypertonie. Aehnliche Versuche von mir, ebenfalls an *Nereis diversicolor*, hatten ein gleiches Ergebnis. Tarussov nimmt aber im Gegensatz zu meiner Deutung der Versuchsergebnisse an, dass der unter dem Einfluss des Aussenmediums veränderte Wassergehalt der Gewebe (eine Hydratation bzw. Dehydratation des lebenden Kolloids) die Ursache der Atmungsänderung sei. Nach meinen Resultaten trifft diese Erklärung nicht das Richtige. Ich fand nämlich, dass bei *Nereis* nach Ueberführung in verdünntes Seewasser (aus 33 v.T. in 9 v.T. Salzgehalt) die Atmung während der ersten drei Stunden um 8·3 Prozent gesteigert ist. Diese Steigerung hält aber auf die Dauer nicht an; nach 38 Stunden zeigt der Sauerstoffverbrauch nicht nur keinerlei Erhöhung, sondern ist sogar um 19·6 Prozent unter den Ausgangswert gesunken, obgleich der Wassergehalt der Gewebe zu dieser Zeit den Anfangswert noch bedeutend übersteigt (s. Tab. XXVII).

Tabelle XXVII. *Nereis diversicolor* (nach Schlieper).

Sauerstoffverbrauch	Aufenthaltsdauer in dem Versuchsmedium	Salzgehalt des Versuchsmediums (v.T.)
100	—	33
108·3	1-3 Stunden	9
80·4	38-40 "	9

Gewicht	Aufenthaltsdauer in dem Versuchsmedium	Salzgehalt des Versuchsmediums (v.T.)
100	—	33
173	3 Stunden	9
182	21 "	9
152	46 "	9

Der Wassergehalt der Gewebe kann also nicht die primäre Ursache der geschilderten Änderung in der Atmungsintensität sein, viel wahrscheinlicher erscheint mir auch hier die Annahme einer aktiven osmotischen Resistenz und einer dadurch bedingten Steigerung des Sauerstoffverbrauches während der ersten Stunden nach der Ueberführung in das hypotonische Medium. In guter Uebereinstimmung mit dieser Deutung stehen auch die Ansichten E. Skujins (1927) über die osmotische Resistenz der Erythrocyten. Dieser Autor fand, dass die roten Blutkörperchen in anisotonischen Lösungen bestimmter Konzentration imstande sind, eine gewisse osmotische Druckdifferenz aufrecht zu erhalten. Er nimmt an, dass dieses auf

Grund osmotischer Arbeitsleistung geschieht und glaubt ebenfalls, dass die Energiequelle hierzu in den Oxydationsprozessen der lebenden Zelle zu suchen ist. Er fand dementsprechend "bei Zuständen, wo der Sauerstoffverbrauch gehemmt ist, bei Sättigung der Blutkörperchen mit Kohlensäure und beim Narkotisieren der Tiere während der Blutentnahme einen geringeren Abstand vom Gleichgewicht (was eine geringere Arbeit der Zelle bedeutet) und eine verminderde Resistenz." Skujin weist ausserdem darauf hin, dass die kernhaltigen Vogelblutkörperchen und junge Erythrocyten, die einen intensiveren Sauerstoffverbrauch haben, resistenter gegen hypertonische Salzlösungen sind.

Weit mehr weisen aber eine Anzahl biologischer Beobachtungen auf das Vorhandensein einer Beziehung zwischen Atmung und Osmoregulation bei vielen Wassertieren hin. So hat u.a. Martini (1923) festgestellt, dass die Kiemenlänge bei Aedes-Larven direkt durch den Salzgehalt des Wassers beeinflussbar ist. Er fand durchweg die Kiemen dieser Larven in Salzwasser kürzer als in Süßwasser. Vogel (1927) fand, dass die Kiemen der an der Mittelmeerküste in Meerwasser vorkommenden Culiciden-Larven—ganz im Widerspruch zu dem Befund an Süßwassertieren—zu kleinen kugelförmigen Anhängen reduziert sind. Thienemann (1928) stellte fest, dass der Schizopode *Mysis relicta* in Norddeutschland nur in verhältnismässig sauerstoffreichem Süßwasser vorkommt, während er im sauerstoffarmen, aber salzreichem Tiefenwasser der Ostsee in Massen anzutreffen ist. Roch (1924) weist darauf hin, dass der Brackwasserpolyp *Cordylophora lacustris* im Binnenland nur in sauerstoffreichem schnellfließenden Wasser vorkommt, während er im Brackwasser überall, auch an stagnierenden oder doch nur schwach bewegten Stellen, anzutreffen ist. Diese Beispiele, deren Zahl sich noch beträchtlich vermehren lässt, sprechen sämtlich dafür, dass das Sauerstoffbedürfnis der betreffenden Tiere im Süßwasser grösser ist als im Salzwasser, und zwar, wie ich in Uebereinstimmung mit dem oben Ausgeföhrtannehmen möchte, auf Grund der im Süßwasser erhöhten osmotischen Arbeitsleistung.—Nur an solchen Stellen im Süßwasser, wo genügend Sauerstoff zur Verfügung steht, ist es dem Brackwasserpolyp *Cordylophora lacustris* möglich, die für seine Existenz notwendige Hypertonie des Innenmediums gegenüber dem Süßwasser aufrecht zu erhalten. Nimmt der Sauerstoffgehalt des Wassers ab, so sinkt auch die osmotische Resistenz des Polypen und damit die Molarkonzentration seines Innenmediums, und es treten, soweit ein Leben überhaupt noch möglich ist, Kümmerformen auf.

Es fragt sich allerdings, ob in allen Fällen bei den Süßwassertieren der osmotische Einstrom nur auf Grund einer aktiven osmotischen Resistenz verhindert, bzw. reguliert wird, ob nicht auch in manchen Fällen eine Verminderung der Wasserpermeabilität und damit eine Herabsetzung des osmotischen Einstromes durch Einlagerung wasserundurchlässiger, aber den Durchtritt der Atemgase nicht hindernder Substanzen in die Außenmembranen erreicht wird. Bottazzi (1907), der etwas Derartiges für viele Süßwassertiere annehmen möchte, weist darauf hin, dass die für die Absonderung der mutmasslichen Substanzen erforderliche Zellenarbeit nicht sehr gross sein kann, während die zur Aufrechterhaltung des Konzentrationsunterschiedes zwischen Innen- und Außenmedium notwendige osmotische Gesamtarbeit ohne Zweifel viel grösser sein würde.

VII. SUMMARY.

The body surfaces and the gills of aquatic animals which use dissolved oxygen for respiration are, in general, permeable to water. It is, therefore, necessary to assume mechanisms for controlling the water content of all those forms in which the body fluids have a molecular concentration differing from that of the external medium. This is the case in all freshwater animals and in marine teleosts. These organisms are homoiosmotic, that is, they are independent of the molecular concentration of the external medium. In contrast to them most stenohaline marine invertebrates are poikilosmotic: their body fluids have an osmotic pressure which is the same as that of the external medium. The beginnings of active osmotic control are, however, apparently present even in this group of animals, since the body fluids of various molluscs and decapod crustaceans and of tunicates are frequently somewhat hypertonic to sea water. Mechanisms for osmotic control are undoubtedly present in those euryhaline marine invertebrates (*Carcinus maenas*, etc.), which are actively homoiosmotic in dilute sea water (Schlieper).—Marine teleosts, in contrast to the last two groups of animals, have a lower molecular concentration in their blood than that of the surrounding sea water. It follows that their osmotic control mechanisms must work in the opposite sense. Something of the same nature exists also in certain invertebrates (*Noctiluca*, *Artemia*, *Pachygrapsus*), that is to say, the internal medium is hypotonic to the external.

It has generally been supposed up to the present, since Overton wrote in 1904, that freshwater animals continuously take in water osmotically through the skin, and then pass it out with the aid of their excretory organs (contractile vacuoles, excretory canal systems of flatworms and rotifers, antennary glands, kidneys, etc.). This view is supported by the fact that the urine of the frog (*Rana esculenta*, according to Bottazzi) and that of a river crab (*Potamobius astacus*, according to Herrmann) is hypertonic to the blood. Newer investigations show, nevertheless, that osmotic control in freshwater animals frequently takes place in quite a different manner from this.

Experiments by Adolph (1926) with *Amoeba proteus* demonstrate that the amount of water passed out by the contractile vacuole is independent of the concentration of the external medium. This makes it probable that the animal actively controls the passage of water through its protoplasm. Certain observations of Gruber (1899) and Herfs (1922), according to which Protozoa without contractile vacuoles (the marine form of *Actinophris*, *Opalina*) can be kept alive for some time in pure fresh water, are pertinent in this connection. An undoubted proof that the excretory organs are not concerned in osmotic control is furnished in those cases where the urine (in *Telphusa* and *Eriocheir*) is found to be isotonic with the blood (Schlieper). It has also been shown that the antennary glands (kidneys) of *Carcinus maenas*, an animal that is homoiosmotic in dilute sea water, always produce urine isotonic with the blood (Schlieper, 1929c). The existence in fresh water of certain animals such

as sponges and Hydrozoa, which have no special excretory organs, is in harmony with this fact that animals whose excretory organs are not concerned in osmotic control can nevertheless live in a hypotonic medium (fresh and brackish water).

The kidneys of marine bony fishes are not concerned in osmotic control either, for the urine which they produce is always slightly hypotonic to the blood. It is at present unknown how the molecular concentration of the body fluids is maintained in marine and in freshwater teleosts, although an indication is given by certain experiments of Duval (1925), proving that in the eel (*Anguilla*) the mucus covering of the body has an osmotically protective function. Gueylard (1923) treads quite a new path in her investigations of the viability of the stickleback (*Gasterosteus*) in sea water. According to this worker the proportion of cholesterol to fatty acids in the blood and tissues is concerned in the capacity of this small freshwater teleost to live in sea water. The spleen, which controls the cholesterol content of the organism in *Gasterosteus* according to Gueylard, is supposed to be the actual organ of osmotic control. Further investigations on this line are needed.

In Amphibia the water content of the body is regulated actively by the skin and by the kidneys according to recent investigations by Parnas (1921), Przylecki (1922-4), Wertheimer (1923-4), Bauer (1925) and Adolph (1925-7). The skin continuously transports water and dissolved salts from outside to inside, while the kidneys selectively excrete superfluous water and salts. The osmotic intake of water appears to be of lesser importance than this actively controlled water current through the body.

Numerous biological observations and experiments show that the oxygen requirements and oxygen consumption increase in euryhaline invertebrates as the salt concentration of the external medium decreases. It is assumed that this increased respiration is required for the work done against an osmotic intake of water from the outside (Schlieper, 1929a).

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THE ORIGIN OF THE AUTOMATIC MICROTOME

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(Received May 21, 1930.)

(With One Text-figure.)

VARIOUS incorrect versions of the origin of the automatic microtome having attained publicity from time to time, I thought that it might not be without interest to put the actual facts on record, as they occurred at Cambridge.

In May 1882 W. H. Caldwell, who was investigating the embryology of marine animals by the method of embedding in paraffin and cutting sections by hand, noticed that, if the paraffin was of the right consistency in relation to the temperature at which it was cut, the section adhered to the razor at the sharp edge of the blade, and did not adhere elsewhere. By cutting a second section without moving the first from the razor, the second section welded itself to the first and pushed it across the razor.

On cutting a third section under the same conditions the same phenomenon occurred, and so on, indefinitely, with the result that a ribbon of sections could be formed. When using the instrument then in vogue for section cutting, it was difficult to make a ribbon composed of more than a few sections owing to irregularity of the cutting. This instrument was simply a glass plate with a central hole through which a brass tube could be pushed up by means of a micrometer screw. The brass tube carried a lump of paraffin on to which a piece of paraffin containing the embedded object could be melted. In trying to cut thin sections, however, there was difficulty in pressing the blade of the razor on the glass plate whilst sliding it over the section, with a sufficiently uniform force. A very small difference in the pressure on the blade bent it appreciably, with the result that successive sections were not of the same thickness: some might be three or four times as thick as others.

The after-treatment of the sections, which were simply put on a warm glass slide, left much to be desired. The paraffin was dissolved away by some solvent, but there was nothing to hold the different parts of the section of the object together. There was, therefore, a difficulty in that the parts moved relatively to each other, and their exact relation could not be ascertained. This practically always occurred when a coverslip was mounted on the section.

Some time towards the end of May or the beginning of June, in the course of a stroll after hall, Caldwell told me of his discovery in regard to the welding of successive sections, and remarked (or I did—I forget who said it first, but most probably Caldwell) that this discovery made the construction of a section-cutting machine a possibility. We discussed in general terms the sort of machine that should be made,

and decided that it should be a block of metal bored so that a metal rod or tube could be screwed up or down through the block by means of a micrometer screw. The amount through which the tube or rod should be raised at each stroke could be adjusted by the motion of the block itself. This general idea was mainly or perhaps entirely due to Caldwell.

Towards the end of June I accompanied Caldwell to Heligoland, where he went to study the development of the particular embryo he was busy with. I was in Heligoland for something like a fortnight, but I do not think we discussed the machine very much. However, when I left Caldwell in Heligoland to return to Cambridge for the long vacation, we arranged that I was to go ahead and see what I could do in carrying out the general ideas for a machine on which we had agreed. This I proceeded to do, keeping Caldwell informed of what I proposed, but on no occasion did he reply to my letters, so that I received no assistance from him. I proceeded to get out drawings showing the machine substantially as constructed, though, as I was (and still am) a very poor draughtsman, the drawings were redrawn in Prof. Stuart's workshop, where I had arranged that the machine was to be constructed at my expense. The machine was put in hand during the Michaelmas Term, 1882, and completed during the Easter Term, 1883. I took the machine to the Cavendish Laboratory and put it through preliminary trials, driving it by a small water motor that happened to be available. During the time that the machine was under construction I met with a good deal of criticism, particularly from Dew Smith, of the Cambridge Scientific Instrument Co., and Caldwell himself. I remember particularly that Dew Smith's view was that I had once seen a horizontal engine and could not get it out of my head, while Caldwell claimed that he had nothing to do with the construction, that he was not in any way responsible for the machine, and that when it failed, as he expected it to do, it would be my affair entirely. This was perfectly just, because Caldwell had nothing whatever to do with the construction of the machine, for which I was also at that time financially responsible.

As it happened the machine succeeded from the first trial, and I sent over to the Department of Comparative Anatomy, where Caldwell was working, for him to bring over some embedded specimens to cut, as, of course, I had begun by cutting paraffin only. It succeeded beyond expectation, and was then taken over by the Comparative Anatomy Laboratory, which was then under Adam Sedgwick, Prof. F. M. Balfour having been killed in Switzerland in July 1882, while I was at work on the design. The machine remained at work in the laboratory for many years. It was to the best of my recollection still at work in 1898 when I came back from Australia, though in the meantime variations had been brought out. The only biological work I ever did with it at Cambridge was to cut an *Amphioxus* into sections from end to end, making a ribbon some yards long. These sections all being of the desired equality in thickness, which if I remember rightly was about $\frac{1}{4000}$ of an inch, satisfied me that the machine was satisfactory so far as the mechanics and construction were concerned.

The perfection of the sections produced by the machine brought into prominence the difficulty in regard to loose portions of the sections floating away

during mounting, and I set to work to endeavour to get over this difficulty, working in the Caius Chemical Laboratory by permission of M.M.P. Muir, who was then praelector in Chemistry at Gonville and Caius College. Mr Muir kindly allowed me to do pretty well as I liked in the laboratory. After a certain amount (not very much) of work I hit on the method which came to be known as the india-rubber method. This was carried out as follows: A dilute solution of rubber in solvent naphtha or benzene was prepared and poured over the slide on which the section was to be mounted, and allowed to dry. The piece of ribbon was then placed on the slide and put on the top of a water-bath, so that the paraffin melted and the section became firmly cemented to the rubber film. After trying many solvents for the paraffin, subject to the condition that it would not dissolve rubber, I eventually found in the Market Place at Cambridge, where it was used for flares, some hydro-carbon which was known as "naphtha." I presume it was a lighter fraction from shale-oil distillation, though I never knew exactly what it was. This solvent met the case admirably. The paraffin was dissolved away and all parts of the sections left firmly in position. The method was used for some time, but was eventually displaced by what was known as the white of egg method invented at Dohrn's Laboratory at Naples, to which the india-rubber method had been communicated in a letter by Caldwell. The india-rubber method was never largely used, because it appears that there were so many substances which were called "naphtha," that mistakes in purchase arose and solvents were purchased which had the property of dissolving rubber as well as paraffin. I was only in my second year at Cambridge and was not up to investigating the products which were called naphtha so as to give a more precise definition. However, I used the method with perfect success in mounting the series of *Amphioxus* sections referred to above, increasing the length of the glass slides for the purpose, and using slips of mica instead of glass coverslips: that is to say, I was able to get perhaps twenty or thirty sections on to a slide and cover them all with one slip of mica, using Canada balsam. I have some few left, though most of them were given away to people interested.

After the success of the machine had been established, although no publication was made except the addition of a page in the then new edition of Foster and Balfour's *Embryology*, where the method was described, improvements began to appear. The first of these that I have any knowledge of was a machine designed by Horace Darwin, of which five or six were made. This was somewhat on the lines of my machine, but very much more elaborate in regard to the means adopted for giving a fine adjustment to the amount by which the object was moved forward after each cut. For some reason that I do not know it was not a success. In the spring of 1883 I took advantage of a new regulation which permitted candidates for the Science Tripos to spend a period up to one year in work abroad. I was at Strasburg under Prof. Fittig and Prof. Kundt from April 1883 till the end of the semester. When I got back to Cambridge—I presume in the long vacation of 1883—I was shown the machine referred to above. It must have been nearly (or quite) a year before Darwin produced his rocking microtome. This was a machine of much simpler construction than my original microtome, simplifica-

tions having become possible by giving up the cutting of rigorously parallel sections. The sections cut by this machine were, in fact, cut to the arc of a circle. I had been told originally that all sections must be rigorously plane and parallel, and that elaborate means must be made available for orienting the objects to be cut in relation to the plane of the cutting edge of the razor. In the meantime these conditions had been found to be superfluous¹.

The rocking microtome was cheap, small, ingenious and convenient, and I imagine that a large number of them were sold by the Cambridge Scientific Instrument Co. Neither Caldwell nor I ever made any further publication, and indeed I never made much of the whole affair. I did no more work at Cambridge in regard to section cutting, as the whole of my time was spent in the Cavendish Laboratory on other matters.

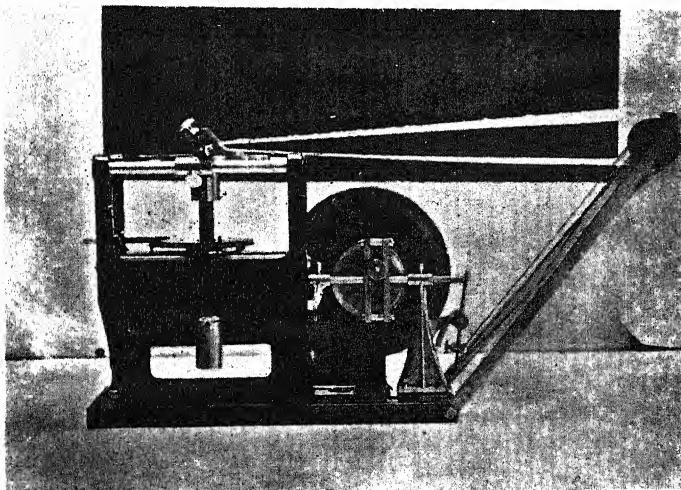
While I was at Strasburg I met a man, whose name I forget, but he was one of the demonstrators in Comparative Anatomy in that University, and was engaged on the embryology of marine animals. I communicated to him the construction of the microtome and the method of section mounting, and received rather a shock when, towards the end of my stay in Strasburg, I found that this man had written a paper in which he published all the information I had given him under his own name, and without any reference whatever to the source from which it had been acquired. No doubt a claim will be put in some day for this invention to the credit of the person in question, or it may have been made already. At all events, several German instrument makers began to make automatic microtomes very shortly afterwards, each of them of different design.

In March 1886 I went to Sydney (Australia) as Professor of Physics in the University. When I had been there a year or two (I forgot the exact date) a workman appeared at my laboratory who stated that he had been the workman formerly employed in Prof. Stuart's workshop and had made my microtome. He had a full kit of tools which he wished to dispose of and he also had a duplicate microtome which he had made at home at the same time that he made mine in Prof. Stuart's workshop, using the same castings, which of course were easily available, the patterns being in existence. This was a very nice machine, and was elaborately nickel plated. The workman, whose name was Ruffett, had developed phthisis, and had been sent to Australia in the hope of recovery. At the time he called on me he was anxious to dispose of his tools and the machine as he was not in a good position financially. In order to help him I bought both the machine and tools and did what I could to find a place well inland where it was hoped he would recover. But he died shortly afterwards. The machine has been in my possession ever since, though I have never used it, having done no embryology since 1883. I believe, however, I did at one time lend it to someone at Sydney who wanted to cut sections.

¹ About 1894 a modification of the rocking microtome was introduced, in which the arm carrying the paraffin block moved in an arc of a circle, the plane of which was parallel to the blade. This produced accurately plane sections, thus getting over the objection that sections cut by the original rocker were curved. At the request of Prof. Adam Sedgwick, Mr (now Prof.) E. W. MacBride undertook, about 1895, a close comparison of sections cut by these two types of microtome. No perceptible difference could be detected between them, even under the highest power of the microscope. (Ed.)

This machine is an absolute duplicate of my original machine, except that Ruffett had thought of a way of getting a finer adjustment than my design allowed in the amount by which the object was advanced at each stroke. It was an ingenious fitting by which two pawls worked together in such a manner that the micrometer screw could be adjusted to give a half-tooth advance. This addition was quite unnecessary, the requisite degree of fineness of motion having been already provided for, but it was quite ingenious.

I am sometimes asked why I did not patent the machine. The answer is that, as it was a machine for the furtherance of scientific research and had no commercial application, both Caldwell and I considered that to take out a patent would have been as improper as it would be for a physician to patent a medical discovery.



Photograph of the duplicate microtome made by Ruffett.

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AT THE UNIVERSITY PRESS

